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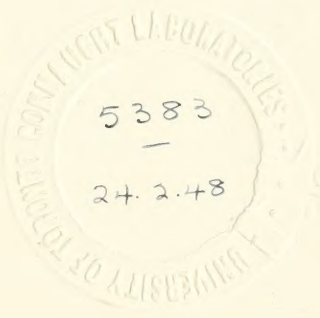

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RANGE EXTENSION OF CEANOTHUS SANGUINEUS.

Oliver A. Farwell.

In the Synoptical Flora and in Piper's Flora of the State of Washington the distribution of *Ceanothus sanguineus,* Pursh is given as from Brit. Columbia to N. California and Idaho. Howell gives the same range but extends it eastward to Montana. Rydberg, in the Flora of Montana, says it occurs only on the western slope of the main range of the Rocky Mountains. Coulter's Manual, 1st. Ed., credits it to the region of the Missouri and its tributaries, but it is not given a place in the 2nd edition. Pursh, who first described it from material collected by Lewis, gives it as "Near the Rocky Mountains, on the banks of the Missouri;" in so far as this statement by itself is concerned, it might mean either the eastern or western slope of the Rockies or both; I have not access to any records that might determine the point in question. The plant, however, has been considered to belong exclusively to the northwestern region west of the main range of the Rocky Mountains. Its discovery, therefore, in the Keweenaw Peninsula, in Michigan, is of more than local interest. I first collected it in fruit in August, 1886, near Copper Harbor. At that time, being young in years and botanical experience, my main object was to make each species I found agree with some one of those enumerated in Gray's Manual; so it naturally found a resting place in the species cover of *C. Americanus,* where it remained forgotten until 1914. Having in that year had occasion to examine critically my material of the eastern species I at once observed that the Keweenaw plant was not *C. Americanus.* An investigation convinced me that it was either *C. sanguineus* or a new species. On a trip to the Lake Superior region early in July of this year (1915) I found the shrub in full bloom. It proved to be *C. sanguineus.* I examined a section of territory about half a mile long by as much wide and found the plant to be quite plentiful; the probabilities are that it may be found over a much larger area. It is found in rocky woods, the rock being

of trap rock formation; the woods are mostly of evergreen trees but have a good sprinkling of oak, birches, willows, poplars, maples and other deciduous trees and shrubs. It is, of course, indigenous to this region. The possibilities of its having been an introduction from west of the Rockies are so remote as to be negligible. Plants from the eastern slope of the Rockies might be introduced to the Lake Superior region by way of the rivers and streams which find an outlet through Lake Winnipeg, Winnipeg River, the Lake of the Woods, Rainy Lake River, and Pigeon River to Lake Superior, but this line of travel would not transport the seed of a plant from the western slope, nor would the Missouri carry the seed from that region to Lake Superior. The same remarks apply to another plant, the *Mimulus moschatus,* Dougl., which is native to the same regions; it may have been introduced further east as an escape from cultivation, but the argument will not apply here, since, so far as I have been able to learn, it was never cultivated in the Copper district of Michigan unless gathered for the purpose from the local native plant. The only probable explanation of such widely separated stations is that the species in preglacial times were more generally distributed, but that the ice of the glacial period destroyed intermediate stations.

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THE ANTIGENIC VALUE OF SPIROCHÆTA HYOS IN COMPLEMENT-FIXATION TESTS ON HOG-CHOLERA SERA.

STUDIES ON HOG CHOLERA.

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No references to laboratory methods for the diagnosis of hog cholera occur in the earlier literature with the exception of that to the use of Bacillus cholera-suis in experimental agglutination tests.[1] Within the last few months reports have been made of the results of some experimental complement-fixation reactions.

Connaway[2] and his associates found that antigen prepared from the blood of pigs suffering from hog cholera was unsatisfactory. Negative results also followed the use of antigens prepared from the spleens and kidneys of virus pigs.

Healy and Smith[3] have published results obtained with an antigen prepared from the mesenteric glands of cholera hogs. This was made by grinding 18 gm. of selected mesenteric-gland tissue with sterile sand. To this, 180 gm. of neutral 1% glucose broth were added, and the mixture allowed to stand for 8 days at 4 C. The results of tests with this material led the authors to conclude that they had obtained "an antigen which shows striking differences in its reaction toward normal hog, rabbit, and cow sera, and hyperimmune hog serum. The antigen is not present in freshly prepared extract of mesenteric glands, but requires a definite period for development; it is not removed from such an extract by passage through an ordinary porcelain filter but is removed by passage through the F bougie. Finally it gradually disappears from the extract." These investigators state that they are seeking to perfect the preparation of the antigen which they have developed, with a view to rendering it more sensitive.

Our study of Spirochaeta hyos,[4] an organism present in the intestinal ulcers, local crypts, and external local lesions of animals suffering from hog cholera, led us to undertake a series of experiments to determine its antigenic value in complement-fixation, a project apparently justified by the results of a rather extensive investigation of this organism.

The complement-fixation test is recognized as one of the most reliable methods of laboratory diagnosis in specific infec-

[1] Giltner: Tech. Bull. Mich. State Exper. Sta., No. 8, 1911; No. 13, 1912.
[2] Bull. Missouri State Exper. Sta., 1914, 131, p. 486.
[3] Jour. Infect. Dis., 1915, 17, p. 213.
[4] King and Baeslack: Jour. Infect. Dis., 1913, 12, p. 307. King, Baeslack and Hoffman: Ibid., p. 365. King and Hoffman: Ibid., 13, p. 463. King and Drake: Ibid., 1914, 14, p. 246, King, Drake and Hoffman: Ztschr. f. Immunitätsf, 1914, 22, p. 347. King and Drake: Jour. Infect. Dis., 1915, 16, p. 1.

tions such as syphilis, gonorrhea, glanders, contagious abortion, and dourine. The test is also of practical use in the standardization of certain antisera, as antimeningococcic and antigonococcic sera, and in checking up the specificity of pathogenic microorganisms.

It is unnecessary to discuss in detail the methods which should be followed in routine complement-fixation work, but for the purpose of recording our results clearly and completely, the following explanations are given.

Apparatus

The tests are carried out in small tubes, 50 mm. in length by 8 mm. in diameter. In addition to the ordinary pipets graduated to 10ths and 100ths, it is a convenience to have small pipets of 0.1-c.c. capacity, made especially for the work from thermometer glass and graduated by mercury into 100 parts, thus affording readings to 0.001 c.c. The solutions used are normal salt solution (0.85 NaCl to 100 c.c. distilled water) and sodium-citrate solution (1% sodium citrate in normal salt solution). Special care is exercised in cleaning tubes, pipets, and other glassware; if chromic-acid cleaning solution is used, the apparatus is very thoroughly rinsed, first in tap water and then in sterile water, before drying and sterilizing, to eliminate inaccuracies due to the presence of foreign matter.

Reagents

Sheep Corpuscles.—Fresh sheep blood is collected in sodium-citrate solution. The red cells are secured by repeated centrifugation and at least 4 washings in normal salt solution. Finally the cells are placed in normal salt solution in 1% suspension. This suspension may be kept for several days in the refrigerator.

Amboceptor.—The amboceptor, hemolytic serum, is obtained from a rabbit previously injected intravenously with varying doses of washed sheep corpuscles. The rabbit should receive injections of 4, 6, and 8 c.c. respectively, at intervals of 7 days. Ten days after the last injection, the rabbit is bled and the serum obtained.

Complement.—The complement, normal guinea-pig serum, is obtained each day in fresh condition and it should be clear.

Antigen.—Spirochetes are obtained by centrifugation from a pure liquid culture of Spirochaeta hyos. The sediment of pure Spirochaeta hyos thus obtained, is washed with normal salt solution, the supernatant liquid removed, and 20 times its volume of absolute alcohol added to the mass of washed spirochetes. The suspension is placed in a mechanical shaker for 24 hours, after which it is incubated at 37 C. for a period of 10 days, being shaken by hand a few times each day during this period. At the end of this time the suspension is diluted with an equal volume of normal salt solution. It is then ready for titration.

In the present work the strain of Spirochaeta hyos used in the preparation of antigen was secured from the intestinal ulcers of Hog 112. This animal was infected with a strain of hog-cholera virus received from

Dr. Moore and Dr. Birch of Cornell University. The antigen was obtained from pure cultures of Spirochaeta hyos grown under oil in ascitic-broth media to which had been added sterile rabbit kidney or testicular tissue.

Serum to be Tested.—The serum to be tested is obtained under aseptic conditions free from red corpuscles and hemolysis. Before use, it is inactivated by heating to 56 C. for 30 minutes in a water bath.

Titration of Reagents

Amboceptor.—The serum from the rabbit immunzed against sheep corpuscles is inactivated by heating in a water bath at 56 C. for 30 minutes. Dilutions of the serum are then made, from 1:100 to 1:2500, and by titration of these with 0.01 c.c. of complement and 1 c.c. of the 1% suspension of washed sheep cells, that dilution is found in which complete hemolysis of the red cells occurs in 1 hour. This represents the amboceptor unit. Twice this amount is used in the test.

Complement.—The complement, fresh normal guinea-pig serum, is titrated against the amboceptor unit thus obtained, for the purpose of determining any variation in the complementary properties of the fresh guinea-pig sera. This titration is carried out each day before the test. The complementary unit is the smallest amount of complement that will completely hemolyze 1 c.c. of a 1% suspension of sheep cells in the presence of 1 unit of amboceptor. For example, 0.005 c.c. complement after 1 hour at 37.5 C. in the water bath, caused partial or no hemolysis; 0.01 c.c., 0.015 c.c., and 0.02 c.c. gave complete hemolysis while the control remained unhemolyzed. Twice the complementary unit is used in the test.

Antigen.—The antigen must be titrated for the presence of hemolysins. Tubes containing different amounts of antigen, 0.005, 0.01, 0.03, 0.05, and 0.1 c.c., respectively, and one containing no antigen, as control, together with 2 units of complement and 1 c.c. of the sheep-cell suspension, are incubated for 1 hour at 37.5 C. in a water bath. None of the tubes should show hemolysis. If all the tubes show hemolysis, either the complement or the cell suspension, or both, are hemolytic. If there is hemolysis in all the tubes except the control tube, the antigen itself is hemolytic and should be discarded.

The antigen in amounts of 0.005, 0.01, 0.02, 0.05, and 0.1 c.c., is titrated against 2 units of complement, 2 units of amboceptor, and 1 c.c. of sheep-cell suspension for the purpose of detecting the presence of any anticomplementary properties. A control tube containing no antigen is also prepared. If there is complete hemolysis in each case after incubation at 37.5 C. for 1 hour, the absence of anticomplementary properties is demonstrated. If hemolysis occurs in the control tube, and inhibition is present in any of the tubes containing the larger amounts of antigen, the amount of antigen used must be less than that causing any inhibition of hemolysis.

For the purpose of determining its antigenic properties the antigen is titrated against a known positive and a known normal, or negative, serum as illustrated in Table 1.

TABLE 1

THE TITRATION OF ANTIGEN FOR ITS ANTIGENIC PROPERTIES

Tube	Amount of Serum, c.c.		Units of Complement	Amount of Antigen,* c.c.
1	Cholera	0.06	2	0.005
2		0.06	2	0.01
3		0.06	2	0.015
4		0.06	2	0.02
5		0.06	2	0.0
6	Normal	0.06	2	0.005
7		0.06	2	0.01
8		0.06	2	0.015
9		0.06	2	0.02
10		0.06	2	0.0

* The antigen, in the largest amount used, should have previously shown no anticomplementary properties.

The tubes are incubated in a water bath at 37.5 C. for 1 hour. Then to each tube are added 1 c.c. of sheep cells and 2 units of amboceptor and the tubes are again incubated in a water bath at 37.5 C. for 1 hour. At the end of the hour, after the sheep cells are added, there should be complete hemolysis in Tubes 5, 6, 7, 8, 9, and 10, but of the first 4 tubes, those containing sufficient antigen to bind the complement, should show complete inhibition of hemolysis. From this titration the amount of antigen necessary to cause fixation of complement is determined and used as the antigenic unit for the actual test.

THE TEST

Table 2 will illustrate the method of conducting the complement-fixation test.

TABLE 2

COMPLEMENT-FIXATION TEST IN HOG-CHOLERA

Tube	1	2	3	4*
Sera used (c.c.) 217 (normal), 207 (known positive), Lapeer (unknown)...	0.02	0.04	0.06	0.06
Antigen (c.c.)	0.01	0.01	0.01	0.0
Complement (c.c.)	0.03	0.03	0.03	0.03
The tubes were incubated in a water bath for 1 hour at 37.5 C.				
Amboceptor (c.c.)	0.04	0.04	0.04	0.04
Cells (c.c.)	1.0	1.0	1.0	1.0
Hemolysis after 40 minutes:				
Serum 217	Complete	Complete	Complete	Complete (control)
Serum 207	++	—	—	Complete
Serum from Lapeer	++	—	—	++

* Since antigen, complement, amboceptor, and cells had been previously tested for anticomplementary properties and hemolysis, the only control of the test was the serum control (Tube 4).

That there was some slight inhibiting action in the case of the Lapeer serum is shown by the failure of Tube 4 to hemolyze completely, but as no hemolysis had taken place in Tubes 2 and 3, a positive reading was given. Of the tubes containing cholera serum (207), Nos. 2 and 3 showed no hemolysis and therefore positive results were recorded.

Hemolysis occurred in all tubes containing Serum 217 (normal); negative results were recorded. It was shown that 0.02 c.c. of serum was insufficient in amount to cause complete complement-fixation—that is, to prevent partial hemolysis.

Results should be read when the action of the controls is complete. If the test shows complete hemolysis in the tubes (see Tubes 1, 2, and 3), it is evident that there has been no fixation of complement by the serum; therefore, the serum is negative (—). If there has been no hemolysis, the complement is bound and the serum is positive (++++). If only a slight degree of hemolysis has taken place, the result is recorded as triple plus (+++). When only about one-half of the cells have hemolyzed, the reading is given double plus (++), while if there are only a few cells left unhemolyzed but still easily seen, a reading of one plus (+) is made. A one plus (+) is interpreted as doubtful; double plus (++), triple (+++), and four plus (++++) as positive.

The readings should be checked up after the tubes have been allowed to stand for several hours; the tubes containing known normal serum should show complete hemolysis, while those representing positive, or cholera, serum, should remain unhemolyzed. The action frequently occurs in less than 1 hour after the cells are added, and in such cases rapid hemolysis may be partially checked by placing the tubes in an incubator or at room temperature instead of in the water bath.

Known positive and negative sera must be subjected to test with the unknown sera to insure proper titration of all reagents. It is obvious that the amounts of sera used should be varied, as, for example, in the test described, in which the smaller amount of serum was found insufficient to cause complete complement-fixation. The amounts of sera used should not be too large on account of the inhibition of hemolysis which might result. This is controlled by Tube 4. Occasionally a serum is found which possesses inhibitory properties. Such a serum must be titrated carefully to determine the amount in which inhibition is negligible.

The Strains of Virus Utilized

Five different strains of hog-cholera virus have been used in conducting these experimental complement-fixation tests. Strain 1 (N. Y.) was received from Dr. Moore and Dr. Birch, of Cornell University. Strain 2 (from Dr. Hauk, of East St. Louis) represented a stock strain built up by mixing together all the strains of virus obtainable. Some of the original strains incorporated in this were secured from field cases, some from the government laboratories at Ames, Iowa, and some from serum-manufacturing laboratories. Dr. Hadley and Dr. Beach, of the University of Wisconsin, furnished Strain 3 (Wisconsin). Strain 4 was secured on October 21 from a cholera-infected herd of hogs at Grosse Isle, Mich. During the week of October 25, hog cholera appeared on the farm of the Michigan State school for feebleminded children, at Lapeer. One test was conducted with a specimen of serum obtained during this outbreak (Strain 5, Lapeer).

Serum was tested from one animal, Hog 63, infected with Strain 6 (Eloise). Hog 63 had received impure cultures of Spirochaeta hyos

isolated from the intestinal ulcers in pigs that had received virus from an outbreak of hog cholera on the farm of the Wayne county hospital, Eloise, Mich.

The following hogs, infected with the different strains of virus, were used in the complement-fixation tests:

Strain 1 New York	Strain 2 St. Louis	Strain 3 Wisconsin	Strain 4 Grosse Isle	Strain 5 Lapeer	Strain 6 Eloise	Unknown Strains
77	187	188	206	Strain	63	192
160	161	203	223	from		189
186	202	217	227	case		220
106	141	205		in		
87	207	216		field		
204	224	218				
208	229	222				
221		214				
230		228				
		215				

SUMMARY OF GENERAL DATA.

With antigen prepared from pure cultures of Spirochaeta hyos there have been conducted 115 complement-fixation tests. Of these, 22 were with normal hog sera from 10 different animals, 1 with serum from an animal which exhibited a reaction only, following inoculation with virus, 6 with sera from 2 convalescent or naturally immune swine, 84 with sera from 34 animals suffering from hog cholera (4 of which had been used as normals), and 1 test each with 2 different lots of hyper-immune serum. Table 3 shows the results obtained.

TABLE 3

COMPLEMENT-FIXATION TESTS WITH ANTIGEN FROM PURE CULTURES OF SPIROCHAETA HYOS

Test	Date of Test	Animal	Date of Collection of Serum	Clinical Condition of Animal	Number of Days After Inoculation	Result of Complement Fixation Test	Remarks
1	10/ 5	Normal A	10/ 5	Normal	..	—	
2	10/ 5	77	3/23	Hog cholera	10	++++	Autopsy on 10th day
3	10/ 7	77	3/23	Hog cholera	10	+++	
4	10/ 5	160	8/18	Hog cholera	9	+	Autopsy on 9th day
5	10/22	160	8/18	Hog cholera	9	++	Autopsy on 9th day
6	10/ 7	Normal B	10/ 7	Normal	..	—	
7	10/ 8	Normal B	10/ 7	Normal	..	—	
8	10/ 8	186	10/ 8	Hog cholera	10	—	Error in technic. See Test 9
9	10/13	186	10/ 8	Hog cholera	10	++++	Autopsy on 16th day
10	10/ 8	187	10/ 4	Hog cholera	17	+++	Autopsy on 18th day
11	10/ 8	188	10/ 3	Hog cholera	12	++	Autopsy on 12th day
12	10/13	Normal C	10/13	Normal	..	—	
13	10/26	Normal C	10/13	Normal	..	—	
14	10/13	106	5/ 6	Hog cholera	10	++++	Autopsy on 10th day
15	10/13	161	8/23	Hog cholera	13	++++	Autopsy on 13th day
16	10/22	161	8/23	Hog cholera	..	++++	
17	10/26	161	8/23	Hog cholera	..	++	
18	11/ 4	161	8/23	Hog cholera	..	++	
19	10/21	Normal D	10/20	Normal	..	—	
20	10/22	Normal D	10/20	Normal	..	—	

TABLE 3—*Continued*

COMPLEMENT-FIXATION TESTS WITH ANTIGEN FROM PURE CULTURES OF SPIROCHAETA HYOS

Test	Date of Test	Animal	Date of Collection of Serum	Clinical Condition of Animal	Number of Days After Inoculation	Result of Complement Fixation Test	Remarks
21	10/21	192	10/20	Hog cholera	13	—	Error in technic. See Test 23
22	10/22	192	10/20	Hog cholera	13	±	Error in technic. See Test 23
23	10/26	192	10/20	Hog cholera	13	++++	Autopsy on 19th day
24	10/21	Hyperimmune serum	.....	.....	..	+++	Received from Michigan exper. station
25	10/21	87	4/15	Hog cholera	10	++++	Autopsy on 10th day
26	10/21	63	1/29	Hog cholera	16	+++	
27	10/29	63	1/29	Hog cholera	16	++++	Autopsy on 16th day
28	10/22	203	10/22	Hog cholera	10	+	See Test 29
29	10/26	203	10/22	Hog cholera	10	++++	Died on 17th day
30	10/22	202	10/22	Hog cholera	10	+	Died on 31st day
31	10/22	Grosse Isle	10/21	Hog cholera	..	+	Secured in field from moribund animal. See Test 32
32	10/26	Grosse Isle	10/21	Hog cholera	..	+++	Secured in field from moribund animal
33	10/29	217	10/29	Normal	..	—	
34	11/ 2	217	10/29	Normal	..	—	
35	11/12	217	11/12	Hog cholera	8	++	Autopsy on 13th day
36	10/29	204	10/27	Symptoms, Temp. 106 F.	9	++	Animal had reaction only
37	11/ 2	204	11/ 1	No symptoms	14	+	Became normal on 13th day
38	11/11	204	11/10	Normal immune	23	—	Exposed with Hogs 208 and 221
39	11/29	204	11/22	Normal immune	34	—	
40	12/ 2	204	11/30	Normal immune	42	—	
41	10/29	Hyperimmune serum	.....	.....	..	+++	Received from Dr. Huff, Sioux City, Iowa
42	10/29	141	9/11	Hog cholera	14	++++	Autopsy on 14th day
43	11/ 2	141	9/11	Hog cholera	..	++++	
44	11/ 2	205	11/ 1	Hog cholera	4	++	Temp. 105. Clinical symptoms 5th day
45	11/10	205	11/ 9	Hog cholera	12	+++	
46	11/11	205	11/ 9	Hog cholera	12	++	
47	11/19	205	11/17	Hog cholera	20	++++	Found dead on 23rd day
48	11/ 2	206	11/ 1	Natural immune	4	—	
49	11/ 2	207	11/ 1	Hog cholera	4	+++	Temp. 105.8. Clinical symptoms. Found dead on 19th day
50	11/ 2	Lapeer serum	10/30	Hog cholera	..	++++	Natural exposure in field
51	11/ 9	216	11/ 3	Normal	..	—	
52	11/10	216	11/ 3	Normal	..	—	
53	11/12	216	11/12	Hog cholera	8	+++	Autopsy on 13th day
54	11/ 4	220	11/ 3	Hog cholera	?	++++	Accidental exposure
55	11/11	220	11/ 3	Hog cholera	?	+++	Found dead on 13th day
56	11/ 4	208	11/ 3	Hog cholera	6	++++	Symptoms
57	11/10	208	11/ 9	Hog cholera	12	++	
58	11/15	208	11/10	Hog cholera	13	++++	
59	11/15	208	11/ 9	Hog cholera	12	++++	
60	11/16	208	11/ 9	Hog cholera	12	++++	
61	11/29	208	11/24	Hog cholera	27	+++	Moribund
62	11/ 4	189	11/ 4	Hog cholera	?	+++	Accidental exposure. Found dead 11/12
63	11/10	Normal E	11/ 9	Normal	..	—	
64	11/11	Normal E	11/ 9	Normal	..	—	
65	11/18	Normal E	11/ 9	Normal	..	—	

TABLE 3—*Continued*

COMPLEMENT-FIXATION TESTS WITH ANTIGEN FROM PURE CULTURES OF SPIROCHAETA HYOS

Test	Date of Test	Animal	Date of Collection of Serum	Clinical Condition of Animal	Number of Days After Inoculation	Result of Complement Fixation Test	Remarks
66	11/11	218	11/10	Hog cholera	6	+	Autopsy on 13th day
67	11/12	221	11/11	Normal	..	—	
68	11/12	221	11/12	Hog cholera	1	—	No symptoms, no fever
69	11/15	221	11/12	Hog cholera	1	—	No symptoms, no fever
70	11/29	221	11/12	Hog cholera	1	—	
71	11/15	221	11/13	Hog cholera	2	—	
72	11/16	221	11/13	Hog cholera	2	—	
73	12/ 1	221	11/13	Hog cholera	2	—	No symptoms, no fever
74	11/16	221	11/14	Hog cholera	3	—	
75	11/16	221	11/15	Hog cholera	4	++	
76	11/18	221	11/15	Hog cholera	4	+++	
77	11/16	221	11/16	Hog cholera	5	+	Slightly inactive, no fever
78	11/19	221	11/16	Hog cholera	5	+++	
79	11/18	221	11/17	Hog cholera	6	+++	Temp. 104.2
80	11/18	221	11/18	Hog cholera	7	—	Error in technic. See Test 81
81	11/19	221	11/18	Hog cholera	7	++++	Temp. 106. Symptoms
82	11/19	221	11/19	Hog cholera	8	++++	
83	11/29	221	11/22	Hog cholera	11	+++	
84	12/ 2	221	11/24	Hog cholera	13	++++	
85	12/ 7	221	11/29	Hog cholera	18	++++	Animal died on 20th day
86	11/15	222	11/11	Normal	..	—	
87	11/12	222	11/12	Hog cholera	1	—	
88	11/15	222	11/12	Hog cholera	1	—	
89	11/15	222	11/13	Hog cholera	2	—	
90	11/16	222	11/14	Hog cholera	3	+++	Temp. 106.2. No clinical symptoms
91	11/16	222	11/15	Hog cholera	4	+++	
92	11/16	222	11/16	Hog cholera	5	+++	Clinical symptoms pronounced
93	11/18	222	11/17	Hog cholera	6	+++	
94	11/18	222	11/18	Hog cholera	7	+	See Test 95
95	11/19	222	11/18	Hog cholera	7	++++	
96	11/19	222	11/19	Hog cholera	8	++++	Found dead on 10th day
97	11/15	Normal F	11/15	Normal	..	—	
98	11/19	Normal F	11/15	Normal	..	—	
99	11/18	223	11/17	Hog cholera	6	—	Field virus, Grosse Isle. Temp. 105.4. Clinical symptoms
100	11/29	223	11/22	Hog cholera	11	+++	Found dead on 21st day
101	11/29	214	11/22	Hog cholera	11	+++	
102	12/ 1	214	11/22	Hog cholera	11	+++	
103	12/ 2	214	11/22	Hog cholera	11	+++	Found dead on 18th day
104	11/29	215	11/19	Chronic hog cholera	8	—	No symptoms. Temp. 104.2
105	12/ 2	215	11/29	Chronic hog cholera	18	+++	Marked symptoms
106	11/29	224	11/19	Hog cholera	8	+++	Autopsy on 25th day
107	12/ 7	227	12/ 2	Hog cholera	5	+	Temp. 104. Slight symptoms
108	12/ 7	227	12/ 4	Hog cholera	7	+++	Temp. 106. Marked symptoms
109	12/ 7	228	12/ 2	Hog cholera	5	+	Temp. 106. Symptoms
110	12/ 7	228	12/ 4	Hog cholera	7	++++	Temp. 107. Symptoms
111	12/ 7	Normal G	12/ 6	Normal	..	—	
112	12/ 7	229	12/ 2	Hog cholera	5	+	No symptoms. Temp. 103.6
113	12/ 7	229	12/ 4	Hog cholera	7	++	No symptoms. Temp. 104.8
114	12/ 7	230	12/ 2	Hog cholera	5	+	No symptoms. Temp. 104
115	12/ 7	230	12/ 4	Hog cholera	7	+++	No symptoms. Temp. 105.4

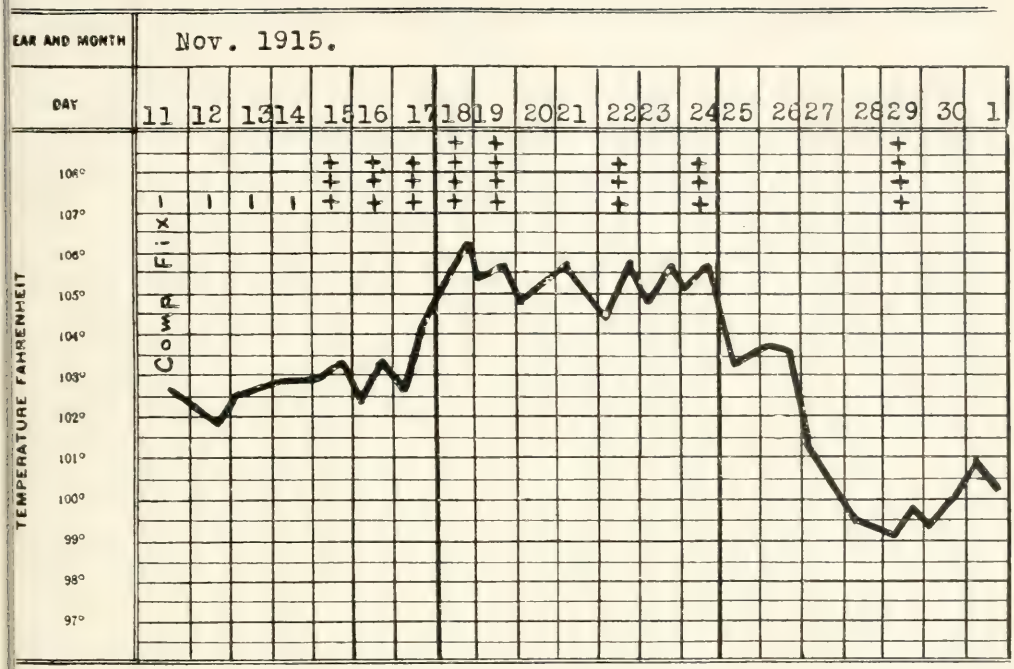


Chart 1. Clinical chart of Hog 221, showing also the time of the appearance of complement-fixation. November 11, intramuscular injection of 2 c.c. of Virus 208, Strain 1 (N. Y.). November 16, slight symptoms. November 18, marked symptoms. November 22, acute hog cholera. December 2, found dead. Examined.

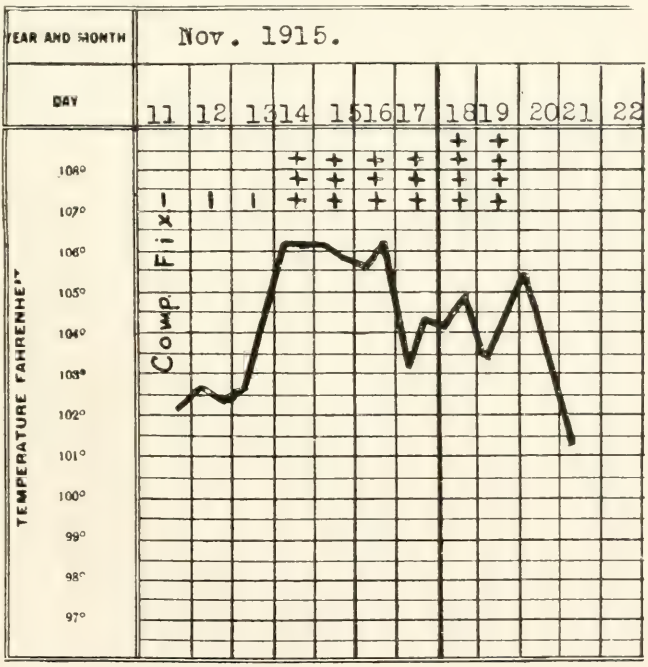


Chart 2. Clinical chart of Hog 222, showing also the time of the appearance of complement-fixation. November 11, intramuscular injection of 2 c.c. of Virus 205, Strain 3 (Wis.). November 15, slight symptoms. November 16, marked symptoms. November 17, acute hog cholera. November 22, found dead. Examined.

These results may be summarized as follows : (1) Hemolysis (—) occurred in all cases in which normal hog sera were subjected to complement-fixation test. (2) Complement-fixation (+) resulted in all tests with sera from cholera hogs, except in Nos. 30 and 66.

THE TIME OF THE APPEARANCE OF A POSITIVE REACTION

In order to determine the number of days after inoculation before complement-fixation appears, daily examinations were made of the sera of two experimentally infected animals (221 and 222). The results obtained are shown in Charts 1 and 2.

With the sera of Hogs 222 and 221, positive serum reactions occurred in 3 and 4 days, respectively. These results corresponded approximately with the apparent periods of incubation, variation in resistance, and types of the disease present in these animals. Hog 222 exhibited a temperature of 106.2° on the morning of the 3rd day, clinical symptoms on the 4th day, and died on the 10th day. Hog 221, the serum of which gave a positive reaction one day later than that of Hog 222, did not show clinical symptoms until the 5th day, or rise of temperature until the 6th day, and lived until the 20th day.

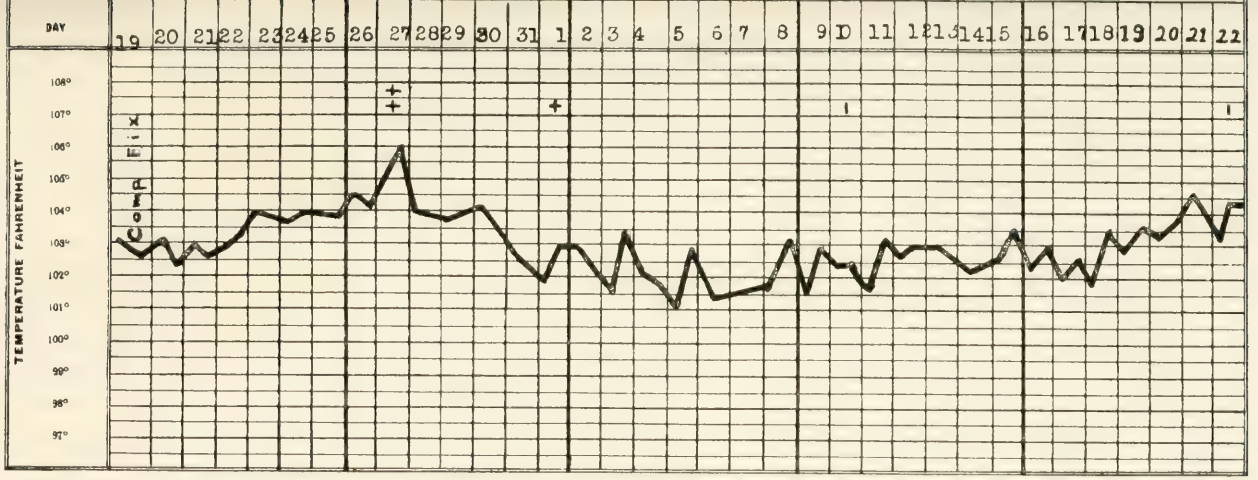
Additional data bearing on this point are presented in Table 4.

TABLE 4

THE TIME OF THE APPEARANCE OF COMPLEMENT-FIXATION

Hog	Incubation Period According to Temperature and Clinical Conditions	Duration of Disease in Days	Type of Disease	Complement-Fixation Test		Results of Subsequent Complement-Fixation Tests
				Day After Inoculation	Result	
205	4 days	23	Subacute	4th	++	12th day, +++; 20th day, ++++
207	4 days	19	Subacute	4th	+++	
227	4 days	..	Acute	5th	+	7th day, +++
228	4 days	..	Acute	5th	+	7th day, ++++
229	7 days	..	Subacute	5th	+	7th day, ++
230	7 days	..	Subacute	5th	+	7th day, +++
208	5 days	27	Chronic	6th	++++	12th and 13th days, ++++; 27th day, +++
218	4 days	13	Acute	6th	+	
223	6 days	21	Subacute	6th	—	11th day, +++
215	9 days	31	Chronic	8th	—	18th day, +++

The results of serum tests applied before symptoms appear or early in the course of the disease, indicate that complement-fixation is coincident with clinical symptoms, and that the time of its appearance depends on the virulence of the infecting material and the individual resistance of the animal.



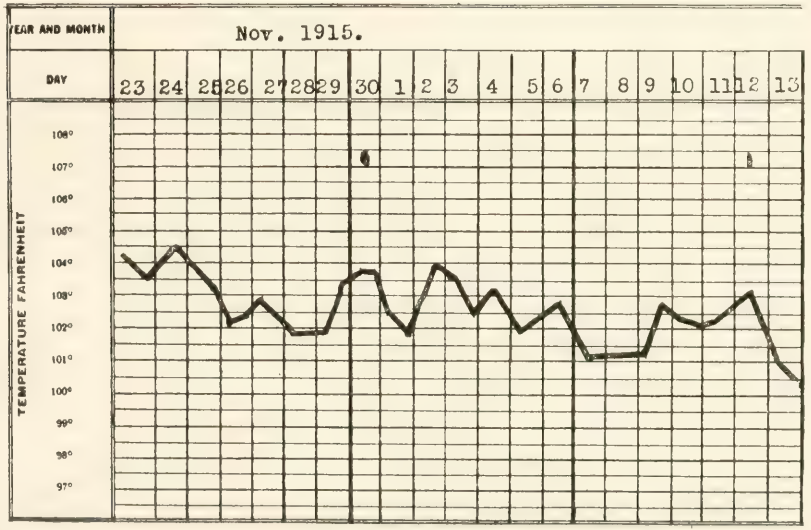


Chart 3. Clinical chart of Hog 204, showing also the results of tests for the duration of complement-binding substances in the blood of Hog 204. October 19, intramuscular injection of 5 c.c. of Virus 160 and of Virus 186. October 25, seemed well. October 27, symptoms. October 31, seemed well. November 3, normal. November 10, normal. November 20, placed in box beside radiator inside. November 24, placed in box outside and bedding kept water-soaked. December 1, cough. December 6, inactive. December 8, seemed normal. December 10, started on salt treatment. December 12, very ill. December 13, moribund.

DURATION OF COMPLEMENT-BINDING SUBSTANCES IN BLOOD OF IMMUNE HOGS

During the course of these experiments one naturally immune hog was found. This animal, Hog 206, was inoculated on October 28 with 2 c.c. of serum from a typical case of hog cholera in a natural field outbreak at Grosse Isle, Mich. On the 4th day after inoculation, serum from Hog 206 failed to fix complement. No symptoms of cholera appeared although the Grosse-Isle serum was virulent for other hogs. Hog 206 was subjected to natural exposure without results, and later was used for other purposes. The serum from this animal continued to be negative in complement-fixation tests. Serum from Hog 204 was also submitted to several tests (see Chart 3).

The results of these tests on the sera of Hogs 206 and 204 indicate that complement-binding substances cease to exist in the blood of hogs when immunity against hog cholera becomes fully established.

CONTROL ANTIGENS

In order that there might be some method of control in this work with pure Spirochaeta-hyos antigen, the following control antigens, prepared according to the method used in making the original spirochete antigen, were tested.

1. B. cholera-suis antigen from a pure culture of B. cholera-suis received several years ago from Theobald Smith.
2. B. Voldagsen antigen from a pure culture of B. Voldagsen received from Dr. Haendel, Königliches Hygienisches Institut, Germany, April, 1914.
3. B. typhi-suis (Glaesser) antigen from a pure culture of B. typhi-suis, also received from Dr. Haendel.
4. Spirochaeta-hyos Antigen 2 from a pure liquid culture from Hog 112 (New York strain).

These antigens were all prepared at the same time and tested with results as given in Table 5.

In these comparative tests with the control antigens, the maximal amounts which would not cause anticomplementary reactions were used. The results show that antigens prepared from pure cultures of B. cholera-suis, B. typhi-suis, and B. Voldagsen, as compared with two lots of pure Spirochaeta-hyos antigen, contain no specific complement-binding properties for hog-cholera serum.

A comparison of Spirochaeta-hyos Antigens 1 and 2, which

were 3 months and 1 month old, respectively, showed the more recently prepared materials to be slightly more active.

TABLE 5

RESULTS OF COMPLEMENT-FIXATION TESTS WITH CONTROL ANTIGENS

Date of Tests	Serum Tested	Amount of Serum c.c.	Antigen	Amounts of Antigen Used, c.c.	Results
11/24	Normal B	0.04	B. Voldagsen	0.005, 0.0075, 0.01	—
11/24	Normal B	0.06	B. Voldagsen	0.005, 0.0075, 0.01	—
11/24	Normal B	0.04	Sp. hyos 2	0.005, 0.0075, 0.01	—
11/24	Normal B	0.06	Sp. hyos 2	0.005, 0.0075, 0.01	—
11/24	Cholera 187	0.04	B. Voldagsen	0.005, 0.0075, 0.01	—
11/24	Cholera 187	0.06	B. Voldagsen	0.005, 0.0075, 0.01	—
11/24	Cholera 187	0.04	Sp. hyos 2	0.005, 0.0075, 0.01	+++
11/24	Cholera 187	0.06	Sp. hyos 2	0.005, 0.0075, 0.01	++++
11/26	Normal B	0.01, 0.02, 0.03, 0.04	B. typhi-suis	0.005, 0.01	—
11/26	Cholera 187	0.01, 0.02, 0.03, 0.04	B. typhi-suis	0.005, 0.01	—
11/26	Normal B	0.01, 0.02, 0.03, 0.04	B. cholera-suis	0.005, 0.01	—
11/26	Cholera 187	0.01, 0.02, 0.03, 0.04	B. cholera-suis	0.005, 0.01	—
11/26	Normal B	0.01, 0.02, 0.03, 0.04	B. Voldagsen	0.005, 0.01	—
11/26	Cholera 187	0.01, 0.02, 0.03, 0.04	B. Voldagsen	0.005, 0.01	—
11/26	Normal B	0.01, 0.02, 0.03, 0.04	Sp. hyos 2	0.005, 0.01	—
11/26	Cholera 187	0.01, 0.02, 0.03, 0.04	Sp. hyos 2	0.005	+++
11/26	Cholera 187	0.01, 0.02, 0.03, 0.04	Sp. hyos 2	0.01	+++
11/26	Cholera 187	0.01, 0.02, 0.03, 0.04	Sp. hyos 1	0.005	+++
11/26	Normal B	0.01, 0.02, 0.03, 0.04	Sp. hyos 1	0.01	+++
11/26	Normal B	0.01, 0.02, 0.03, 0.04	Sp. hyos 1	0.005	—
12/ 2	Normal 204	0.06	Sp. hyos 1	0.01	—
12/ 2	Normal 204	0.06	Sp. hyos 2	0.01	—
12/ 2	Normal 204	0.06	B. Voldagsen	0.01	—
12/ 2	Normal 204	0.06	B. typhi-suis	0.01	—
12/ 2	Normal 204	0.06	B. cholera-suis	0.01	—
12/ 2	Cholera 221	0.06	Sp. hyos 1	0.01	—
12/ 2	Cholera 221	0.06	Sp. hyos 2	0.01	+++
12/ 2	Cholera 221	0.06	B. Voldagsen	0.01	—
12/ 2	Cholera 221	0.06	B. typhi-suis	0.01	—
12/ 2	Cholera 221	0.06	B. cholera-suis	0.01	—
12/ 2	Cholera 221	0.06	Sp. hyos 1	0.01	+++
12/15	Cholera 215	0.06	Sp. hyos 2	0.02	+++
12/15	Cholera 215	0.06	Sp hyos 1	0.02	++
12/15	Cholera 215	0.06	B. Voldagsen	0.02	—
12/15	Cholera 215	0.06	B. typhi-suis	0.02	—
12/15	Cholera 223	0.06	B. cholera-suis	0.02	—
12/15	Cholera 223	0.06	Sp. hyos 2	0.02	++
12/15	Cholera 223	0.06	B. Voldagsen	0.02	—
12/15	Cholera 223	0.06	B. typhi-suis	0.02	—
12/15	Cholera 223	0.06	B. cholera-suis	0.02	—
12/ 3	Normal 204	0.04	Sp. hyos 2	0.02	—
12/ 3	Normal 204	0.04	B. typhi-suis	0.02	—
12/ 3	Cholera 227	0.04	Sp. hyos 2	0.02	+++
12/ 3	Cholera 227	0.04	B typhi-suis	0.02	—
12/ 3	Early cholera 228	0.04	Sp. hyos 2	0.02	+
12/ 3	Early cholera 228	0.04	B. typhi-suis	0.02	—
12/ 3	Early cholera 229	0.04	Sp. hyos 2	0.02	++
12/ 3	Early cholera 229	0.04	B. typhi-suis	0.02	—
12/ 3	Early cholera 230	0.04	Sp. hyos 2	0.02	+++
12/ 3	Early cholera 230	0.04	B. typhi-suis	0.02	—

CONTROL COMPLEMENT-FIXATION TESTS WITH SERA OF HOGS SUFFERING FROM DISEASES OTHER THAN HOG CHOLERA

In considering the possible specificity of the Spirochaeta-hyos antigen in complement-fixation tests with hog-cholera serum, it appeared necessary to determine the results of the application of the test to sera obtained from hogs suffering from disease processes other than that of hog cholera. The committee on diseases, of the American Veterinary Medical Association, in

August, 1915, reported as follows concerning the differential diagnosis of hog cholera:

Among the diseases or disease conditions that must be differentiated from hog cholera, are parasitism, a form of infectious enteritis, that condition which the U. S. Bureau of Animal Industry calls Salmonellosis and is supposed to be due to the *Bacillus suipestifer,* the so-called swine plague, pneumonia, verminous pneumonia, brine poisoning, acute pericarditis, shoat typhoid, enteritis and poisoning from spoiled foods, soap powders and irritating stock powders, swine erysipelas (which so far as we know does not exist in this country) septicemia, malignant edema, necrotic laryngitis, anthrax, heat stroke, lightning stroke, or sudden death from any cause, and a number of acute febrile conditions, that we have met with in pigs, but so far have been unable to classify.[5]

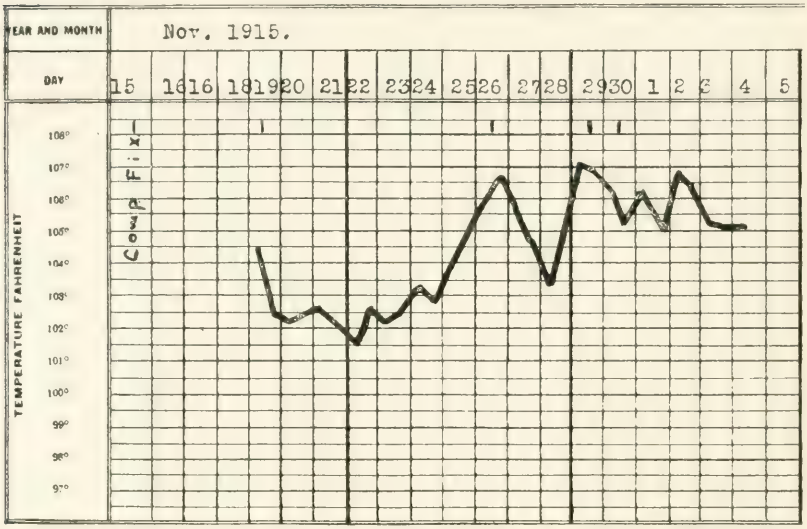


Chart 4. Clinical chart of Hog 225, which had been experimentally infected with Staphylococcus aureus. Results of complement-fixation tests are shown at the top of the chart. November 18, intramuscular and subcutaneous injections of 10 c.c. of a mixed broth culture of Staph. albus, Staph. aureus, and streptococcus. November 19, swelling at sites of injection. November 20, intramuscular injection of 10 c.c. mixed staphylococcus cultures Nos. 10, 15, 16 from hogs, and pyocyaneus (Ward). November 26, animal dull. November 29, ill. December 4, killed and examined.

In this investigation some of the foregoing pathologic conditions have been experimentally produced:

Septicemia.—Hog 225 (see Chart 4) exhibited typical clinical symptoms of septicemia and bacteremia. Fifteen days after the first inoculation the animal, which had been kept under carefully isolated conditions during the experiment, was killed and examined. The animal was not emaciated. There were swelling and induration at the points of inoculation. Lymphatic glands enlarged and hemorrhagic. Lungs contained a few small

[5] Jour. Am. Vet. Med. Assn., 1915, 48, p. 221.

hemorrhagic points and one or two small areas of congestion. Heart, spleen, and liver normal. Kidneys slightly congested and covered with a few petechiae. Ental surface of bladder normal. Intestinal tract normal except for the presence of Ascaris suum and a slight inflammation of the mucous membrane of the large intestine.

Flask broth cultures, made from the heart blood under aseptic conditions, after 24 hours' incubation yielded pure colonies of Staphylococcus aureus in agar transfers.

B. Cholera-Suis Infection.—Hog 231 (see Chart 5) showed the following: Lymphatic glands enlarged but only slightly congested. Both lungs filled with numerous small hemorrhagic areas. Heart and liver normal. Spleen normal in size, but congested in areas and soft in consistency. Kidneys congested and from 1 to 5 petechiae present. Mucosa of large intestine normal except for a few areas of ecchymosis. Ental surface of bladder normal. B. cholera-suis recovered in pure culture from the heart blood.

Anthrax.—On November 20, Hog 226 was injected intramuscularly with 2 c.c. of a 24-hour broth culture of B anthracis. On November 22, as this animal showed symptoms of illness and a temperature of 104.4°

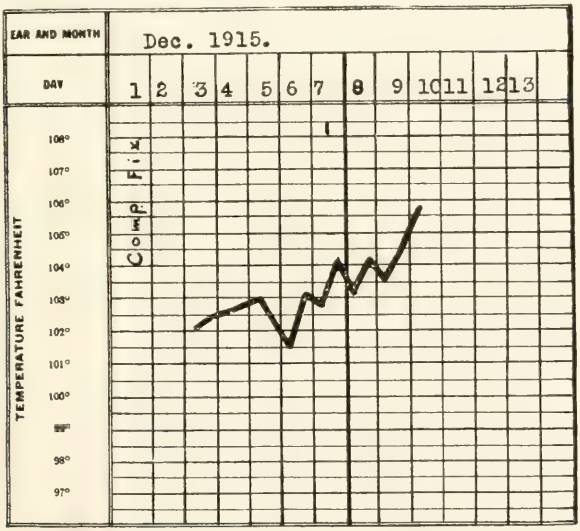


Chart 5. Clinical chart of Hog 231, which had been experimentally infected with B. cholera-suis. Results of complement-fixation tests shown at the top of the chart. December 1, intramuscular injection of 11 c.c. of a culture broth of B. suipestifer 052 (Theobald Smith). Given 5 c.c. of same cultures orally. December 7, ill. December 9, anorexia. December 10, very ill; killed and examined.

a specimen of serum was collected and submitted to complement-fixation test, with negative results. On November 24, the condition of Hog 226 was normal and subsequent injection with massive doses of B. anthracis demonstrated that the animal had acquired active immunity.

Hog 233 was injected with 20 c.c. of a virulent broth culture of B. anthracis on December 9. The animal, moribund on December 12, was

killed; specimens of heart blood were secured for serum tests and cultures, and an autopsy made. Edematous swelling at point of inoculation. Enlarged engorged spleen. Kidneys congested. Lymphatic glands enlarged and hemorrhagic. Cultures from heart blood yielded pure B. anthracis. Complement-fixation tests of the serum from this animal with Spirochaeta-hyos antigen resulted negatively.

Ghon-Sachs-Bacillus Infection.—Ten cubic centimeters of a deep glucose-agar culture of the Ghon-Sachs bacillus[6] (original furnished by Dr. K. F. Meyer) were injected intramuscularly into Hog 232 on December 9. The following day the site of injection was surrounded by a large tender edema, the animal was inactive, and the temperature had risen to 104.6°. A specimen of serum was secured from Hog 232 on December 10. The complement-fixation test resulted negatively. The 4th day after inoculation the swelling decreased, temperature fell to 102°, and the animal resumed normal condition.

Brine or Salt Poisoning and Pneumonia.—Hog 204, immune to hog cholera (see Chart 3), was utilized for experimental brine poisoning. It will be noted in the clinical chart for this animal that an attempt was made to produce pneumonia. From November 20 to 24, Hog 204 was kept beside a warm radiator, after which it was exposed to cold and dampness. During this period the animal developed a cough, irregular temperature, and chills. Specimens of serum collected on November 22 and November 30 failed to show complement-fixation with the spirochete antigen.

From December 10 to 12, Hog 204 was given salt. On December 12 the animal showed pronounced symptoms of brine poisoning, and on December 14 death occurred. A complement-fixation test with the serum secured December 12 resulted negatively. The findings at autopsy were as follows: Animal emaciated. Lungs congested, showing large areas of gray hepatization. Lymphatic glands enlarged but not hemorrhagic. Pericarditis present. Heart enlarged. Liver mottled, engorged with blood, and enlarged. Spleen and kidneys normal in appearance. Intestinal mucosa congested.

These results show that antigen prepared from a pure culture of Spirochaeta hyos possesses no complement-binding properties when brought into contact with the sera of hogs suffering from septicemia (Staph. aureus), from infection with B. cholera-suis, B. anthracis, or the Ghon-Sachs bacillus, from brine poisoning, or from pneumonia by natural exposure.

DISCUSSION

In reviewing the method used in these complement-fixation tests, and in attempting to emphasize the importance of careful technic and proper controls, we wish to quote the following from a recent article by Watson:[7]

[6] Meyer: Jour. Infect. Dis., 1915, 17, p. 458.
[7] Parasitology, 1915, 8, p. 156.

The successful practice of the complement-fixation test depends mainly upon the preparation and use of powerful reagents, their specificity and the accurate determination of their relative values, the fixing of standard doses wherever possible, and a constant, uniform technique and method of procedure.

Close familiarity with the activity of the reagents is essential for the best results.

Stock reagents should be prepared in quantities calculated to meet all requirements for as long a time as the activity of the reagents remains practically constant. Thus: sufficient hemolytic serum for six months' work; antigen to suffice for one month's work; fresh red cell suspension once a week; fresh complement daily or on alternate days, or as needed. It is advisable to use the blood of two sheep for sensitizing rabbits and to use the red cells of the same sheep for the hemolytic system.

The following points of extreme importance will bear repetition:

1. The amount of red cells in suspension must be very accurately measured and the standard never varied.

2. The use of the least possible amount of complement which with two units of hemolytic serum causes complete hemolysis of red cells.

3. The use of twice the amount of antigen which with a dourine antibody unit is necessary to fix the complement, provided the same amount of antigen alone has no inhibitory action.

4. Careful control of the inactivation of suspected area by known positive and known negative sera.

5. Control of the diagnostic tests by a series of known positive sera, each having an antibody unit of different value, high to low.

This quotation applies equally well to the subject of complement-fixation in hog cholera except for the antibody unit of the serum, the determination of which has not been attempted.

CONCLUSION

Antigen prepared from Spirochaeta hyos grown in pure culture possesses well-marked specific complement-binding properties.

This antigen, when brought into contact with the sera of experimentally infected cholera hogs, produces initial complement-fixation at a period coincident with completion of the incubation period as observed in clinical conditions and thermal reactions. The specific properties of the antigen are shown to be present until death of the animal, or until active immunity is fully established.

The sera of normal hogs and those experimentally infected with B. cholera-suis, the Ghon-Sachs bacillus, B. anthracis, Staph. aureus, and also the serum of one hog which was the subject of

pneumonia from natural exposure, and which died from acute brine poisoning, all reacted negatively when tested for complement-fixation with Spirochaeta-hyos antigen.

We believe that, with the observance of proper technic, the results recorded here can be duplicated without difficulty and that the method may be used to practical advantage as a reliable accurate means of laboratory diagnosis of hog cholera. Furthermore, the results of these experiments support our former conclusions that Spirochaeta hyos merits serious consideration as an organism possessing specific pathogenic properties in relation to hog cholera.

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BACTERIOLOGIC FINDINGS IN OZENA.

By Herbert C. Ward.

(From the Research Laboratory of Parke, Davis & Co., Detroit, Michigan.)

Inquiry into the bacteriology of ozena has engaged the attention of a number of workers during the past 25 years.

In the early eighties of the last century, investigations on the part of Fränkel, Löwenberg, Friedländer, Weichselbaum, Paulsen, and particularly the work of Abel,[1] furnished the interesting suggestion that ozena was due to a bacillus called by Abel "Bacillus mucosus Ozaenae." This organism was recognized by him to be identical with Löwenberg's bacillus,[2] and closely related to Friedländer's pneumobacillus. Abel found it present in 100 cases of ozena, while in 250 normal controls he failed to discover it once. Paulsen[3] isolated B. mucosus ozaenae in 51 cases; Strazza[4] in 25; Löwenberg[2] in 16; Marano[5] in 10; Thost[6] in 12 of 17; and Hajek[7] in 7 of 10.

In contrast with these findings there is a marked absence of Abel's bacillus in infections other than ozena. Abel and Paulsen, reporting their own study of several hundred examinations and taking into account all work published up to that time, estimated that B. mucosus occurred in only 1% of nasal cases and in about 3% of oral cases. Netter,[8] in an examination of the sputum of 165 healthy persons, found Abel's bacillus 3 times. Rosenthal[9] in 14 cases found it in 3, these being tuberculous. Krause[10] studying 30 cases of influenza discovered it once. Kowalski likewise obtained it once in 16 cases of influenza. Podbielski[11] in 50 oral examinations found it once. Besser[12] investigating the secretions of 81 nasal cases obtained it twice. Wright[13] reports it totally absent in 10 cases. Paulsen in his study of 27 normal and 24 acute catarrhal conditions failed to obtain a single culture. When Nicolle and Herbert[14] conducted their investigation of 1,600 cases of angina, this bacillus was found in pure growth in the mouths of 6 patients.

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[1] Ztschr. f. Hyg. u. Infektionskrankh., 1896, p. 89. Centralbl. f. Bakteriol., 1893. 31, p. 161.

[2] Deutsch. med. Wchnschr., 1885, 11, p. 23.

[3] Centralbl. f. Bakteriol., 1890, 8, p. 344.

[4] Primo Congreso della societa italiana die Laringologia, 1893.

[5] Centralbl. f. Bakteriol., 1890, 8, p. 179.

[6] Deutsch. med. Wchnschr., 1886, 12, p. 161.

[7] Berl. klin. Wchnschr., 1888, 25, p. 659.

[8] Cited by Baumgarten's Jahresbericht, 1890, p. 81.

[9] Ein Beitrag zur Kenntniss der Bakterienflora der Mundhohle. Dissertation. 1893.

[10] Virchow's Arch. f. path. Anat., 1891, 85, p. 325.

[11] Centralbl. f. Bakteriol., 1891, 9, p. 617.

[12] Ibid., 1890, 7, p. 151.

[13] New York Med. Jour., 1889, 50 p. 92.

[14] Ann. de l'Inst. Pasteur, 1897, 11, p. 867.

Stein[15] in 51 typical cases of ozena, recognized Abel's bacillus in 44 cases. His 35 healthy controls yielded but 2 cultures. Surveying the entire field he reported the presence of bacterial groups as follows:

Bacillus mucosus ozaenae (in ozena 44)	47
Staphylococcus albus and aureus	43
Diphtheroid bacilli (in ozena 25)	37
Micrococci, unidentified types	29
Streptococci and pneumococci	11
B. pyocyaneus	3
Bacillus mucosus-ozaenae (uncertain type)	4
Spore-bearing bacilli	2
Perez bacillus	0

Cobb and Nagel[16] in an extensive study of 90 cases of ozena, with special care obtained B. mucosus ozaenae in every examination. Page[17] isolated Abel's bacillus in 2 cases and supplied fermentation tests for its identification.

Baurowitz[18] reported Abel's bacillus present in 12 patients with ozena. Seven of these showed pronounced symptoms and the fetor was marked. From 6 he obtained organisms capable of developing the characteristic ozena odor while in culture.

A little later, Perez[19] reported his bacteriologic findings in 63 cases, 22 of which presented typical symptoms of ozena. The organisms are arranged in the order of their numerical occurrence:

Staphylococcus albus and aureus	37
Pseudodiphtheria varieties	27
Löwenberg-Abel bacillus (in ozena 17)	25
Perez coccobacillus (in ozena 8)	8
Coli-group strains	7
Streptococci	5
Pneumococci	5
B. pyocyaneus	5

Perez emphasized the finding of a bacterial species which was capable of reproducing the ozena odor while in culture media. He demonstrated that this organism, named by him Coccobacillus foetidus Ozaenae was pathogenic for rabbits, while Abel's bacillus was not. Furthermore, its action was selective, showing affinity for the nasal mucosa, and symptoms developed which simulated those in man. Injected rabbits developed acute and sometimes even chronic conditions, and Perez was able to recover his bacillus from the animal's nasal discharges and to identify it. No such results were to be obtained with Abel's bacillus.

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[15] Centr. ... f. Bakteriol., 1900, 28, p. 326, 769.
[16] Ann. Otol. Rhinol. and Laryng., 1912, 21, p. 46.
Jour. Med. Research, 1912, 26, p. 189.
[17] Centr. f. Bakteriol., 1895, 18, p. 749.
[18] Ann. de l'Inst. Pasteur, 1899, 13, p. 993.

Hofer[20] in a careful investigation of 14 cases isolated Perez' bacillus in 57%, and Abel's bacillus in 86%. Hofer verified Perez' work with rabbits, concluding with him that his organism was the most important factor in ozena.

Recently a few reports have appeared in this country. Guggenheim[21] reviewed Hofer's work and noted the finding of Perez' bacillus in a few cases of ozena. His preliminary animal experiments were also in accord with Hofer's. Horn[22] at a recent meeting of the American Medical Association read a paper on the etiology of ozena, in which he favored Perez' bacillus as being the most important etiologic factor.

All these investigations show that the most important bacterial organisms come under two bacteriologic groups: the Friedländer group, represented by Abel's bacillus, and the Bacillus-suipestifer group, represented by Perez' bacillus. In order to extend the present bacteriologic survey in this field, I have studied 50 well-authenticated cases of typical atrophic rhinitis, the majority of which presented ozena conditions.

Pure cultures of the important species were identified biochemically. Special attention was given to the strains obtained from the first few cases. When experience had determined the leading cultural or morphologic characteristics, a less extensive series of tests sufficed for identification purposes. Sero-biologic titrations were employed in the admittance of new strains to the established class, especially in the case of Perez' bacillus. Dr. W. R. Murry and Dr. W. P. Larson, of Minneapolis, by sending me one of Hofer's original cultures, enabled me to establish identification, and so directly correlate our investigations. Pathogenic action was determined with rabbits, these having been reported as promising a differential value.

TYPES OF BACTERIA AND THEIR RELATIVE FREQUENCY IN ATROPHIC RHINITIS.

A tabulation of the results shows the relative frequency with which the members of the various bacterial groups appeared. The microscopic picture presents the best record within our reach and is therefore to be preferred, were it not also true that morphologic uniformity prevents identification. The cultural picture is less representative, unimportant species becoming prominent and valuable ones disappearing. It seemed desirable, therefore, to combine the findings obtained by the two methods of study.

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[20] Wien. klin. Wchnschr., 1913, 25, p. 1011.

[21] Interstate Med. Jour., 1915, 22, p. 2.

[22] Jour. Am. Med. Assn., 1915, 65, p. 788.

The bacteriologic groups are arranged in the order of their frequency in the 50 cases.

Diphtheroids	43
Staphylococci, all varieties	37
B. mucosus-capsulatus	30
Perez' bacillus	22
Streptococci, all varieties	20
B. proteus, all varieties	20
Pneumococci	20
B. pyocyaneus	12
Spore-bearing types	10
M. catarrhalis	5
B. coli types	5
B. influenzae	4

In addition to these predominant forms, there appeared a variety of other organisms whose occurrence was limited, or difficult to verify culturally.

The diphtheroid group appeared most frequently, a fact in accord with the findings of the earlier workers. The special morphology of this group, together with the use of blood-serum media, explains its predominance.

The micrococci, second in order of frequency, demonstrate that conditions are favorable for their growth and action. Both Perez and Stein have placed this group of bacteria second in their tables.

Abel's bacillus appeared 30 times. The presence of this organism has been considered as typical of bacteriologic conditions in ozena, even by those unwilling to ascribe to it any etiologic significance. The cultures on the agar plates were often pure, while the microscopic picture of the same material revealed a variety of forms. Cobb and Nagel found this bacillus present in 100% of their cases.

Perez' bacillus, seen first by Horowitz, appeared 22 times in my series. It was identified by culture 15 times.

Perez reports this bacillus as a small, easily stained, gram-negative coccobacillus, showing extreme morphologic variations. It is nonmotile and forms shining transparent colonies. Gelatin is not liquefied or lactose fermented. Milk is not coagulated. Potato shows an abundant yellowish moist growth. It is pathogenic for guinea-pigs, mice, pigeons, and rabbits. All broth cultures give off a characteristic fetid odor, especially in the presence of albuminous media.

Specific agglutinins were easily produced. On the receipt of

Hofer's culture, a vaccine was prepared and injected subcutaneously into rabbits. Agglutinins appeared following the second injection, and after 4 doses agglutination of the Hofer strain was positive in a dilution of 1:3000.

Perez reported the production in broth of a peculiar odor, developing more or less strongly on incubation, which he used for the identification of his bacillus. With many of our cultures no odor was produced in the original broth. In addition, numerous observations lead us to believe that there exists a small group of bacilli closely allied to Perez', but characterized by motility, and a lack of odor in broth cultures. Also, these strains are unaffected by the Perez agglutinins. Otherwise they appear culturally identical. All of our strains which were admitted to the Perez group developed this odor, though it appeared at times to be transient in character. The nature of this decomposition was studied in a few instances. Shurly[23] believed that the chemical character of the secretions and the histologic elements present determined the character of the decomposition. I consider that this stench is produced by the activity of a specific proteolytic enzyme. To test this property Perez used serum broth; Hofer, broth; Guggenheim, albuminized media. In order to gauge this activity, tests were made for the presence of methyl-mercaptan, employing the isatin sulfuric acid method.[24] Flasks of plain broth were inoculated severally with pure cultures of Perez' bacillus, proteus, diphtheroid, Abel's bacillus, and several others. Five cultures of Perez' bacillus produced in broth a strong hydrogen sulfid and mercaptan content; 4 cultures of Abel's organism produced no hydrogen sulfid and no mercaptan; 4 cultures of B. proteus produced strong hydrogen sulfid and strong mercaptan; all other tests were negative. These results suggest that the character of the ozena odor is determined by the presence of some of the well-known organic sulfur compounds, especially the mercaptans.

The streptococci appear in the table fifth in order. No special consideration will be given them, since they rarely occurred as the predominating type in the original microscopic pictures.

Sixth in order of isolation stands the proteus group. Just how important this ubiquitous saprophyte may be, it is hazardous to state. Other workers have neglected it entirely. Hajek's organism probably belonged to this group.

It is a luxuriant saprophyte and produces a larger growth and more active enzymic changes in all protein solutions than does Perez' bacillus. It also produces a most nauseating odor in pure culture. This odor is to be distinguished from that produced by other organisms by its suggestive mouselike quality.

[23] Diseases of the Nose and Throat, 1907, p. 376.

[24] Bauer: Ztschr. f. physiol. Chem., 1902, 35, p. 346.

PATHOGENIC ACTION OF PEREZ' BACILLUS.

The work reported in the last division of this paper verifies the finding of Perez and Hofer. They proved that this bacillus was pathogenic for laboratory animals; large doses produced death in rabbits, small doses produced marked reaction, seen in the nasal membranes and in the production of excessive mucopurulent secretions, sometimes developing into a chronic condition.

In this study, 28 healthy rabbits received intravenous injections of the Perez culture; 7 rabbits received cultures of B. proteus; 6 of Abel's bacillus, together with 3 cultures of an organism known as Bacillus bronchisepticus, these last serving as controls.

Of the 28 animals injected with Perez' bacillus 12 died—7 after 24 hours, 1 after 3 days, 2 after 3 weeks, and 2 after 4 weeks. All showed a rise of temperature, increased nasal discharge, and in those animals dying last, caking about the nostrils. Corrosions at the site of inoculation frequently developed, and in the chronic cases emaciation was marked.

The records of the animals receiving B.-proteus injections show that of 7 under test 3 died, presenting conditions at autopsy similar to those observed in the Perez rabbits. After injection all the animals showed pronounced nasal reactions and the organism was recovered from the discharges in 2 cases. Those animals receiving the bronchisepticus cultures developed in 2 instances all the symptoms of snuffles. This condition lasted for several days, recovery following in both instances. In contrast to the foregoing the rabbits which had received the culture of Abel's bacillus showed no reaction and suffered from no infection.

Future study offers interesting possibilities of reversing the weight of etiologic evidence which is at present ascribed to Perez' bacillus. One of the conclusions arrived at by Herbert and Nicolle in their study of angina was that Abel's bacillus held an important etiologic relationship to certain infections. They stated that it was pathogenic, capable of producing acute and chronic conditions. Allen[25] has made the repeated observation that the Friedländer bacillus is to be found associated with chronic conditions. Babes,[26] in his study of rhinoscleroma, suggested the pathogenic relationship of this bacillus to that disease. Abel's bacillus, the original Friedländer's bacillus, and Frosch's bacillus are considered by Perkins[27] to represent but varieties of one group, under the title of B. mucosus-capsulatus.

If the two organisms are compared on the basis of their patho-

[25] The Bacterial Diseases of Respiration, and Vaccines in their Treatment, 1913.

[26] Kolle and Wassermann, Handb. d. pathogen. Mikro-organismen, 1913, 5, p. 1237.

[27] Jour. Infect. Dis., 1904, 1, p. 241.

genic action in experimental animals, the etiologic importance of the Perez organism seems pronounced. However, when Hofer selected control organisms for his experimental work, he unfortunately chose cultures having practically no value as regards the main point of his work—to prove specific action on the part of Perez' bacillus. His results, together with those of others, would have been much more conclusive had he used organisms found in ozena exudates and infections common to his animals.

It has long been observed that rabbits and other laboratory animals are very susceptible to a form of infection spoken of as "snuffles." Dogs, likewise, are known to succumb to a form of rhinitis presenting symptoms similar to those of ozena in man. In a few reports the idea that the human infection has arisen from the canine is set forth, though no proof has, as yet, been offered in support of this assumption. Recently, Ferry,[28] McGowan,[29] and Torry,[30] working independently, discovered and proved the relationship of a small bacillus known as B. bronchisepticus to all cases of canine distemper.

B. bronchisepticus is capable of producing in rabbits a type of rhinitis difficult to be distinguished clinically from the pathologic condition produced by Perez' bacillus. In four instances rabbits which had received intravenous injections of Perez' bacillus and organisms not Perez' developed "snuffles." From nasal discharges almost pure cultures of B. bronchisepticus were isolated. The close cultural characteristics of these two organisms, especially if differential study is not carried out in detail, might be easily overlooked.

FREQUENCY OF OZENA TYPES IN OTHER CONDITIONS.

In order to measure the comparative values of the Abel, Perez, and proteus organisms in ozena, a control study was made (in co-operation with Dr. W. H. Price, health officer of Detroit, and Dr. D. M. Griswold, in charge of the laboratories) to determine their presence in other conditions. A bacteriologic survey, including 1,400 examinations of cultures from suspected cases of diphtheria, was made. Attention was given to the relative per-

[28] Ibid., 1911, 8, p. 399.
[29] Jour. Path. and Bacteriol., 1911, 15, p. 381.
[30] Jour. Med. Research, 1913, 27, p. 291.

centages of cases in which the leading types appeared. The results were as follows:

Abel's bacillus	3 %
Perez' bacillus	0.1%
B. proteus	1 %

When we contrast with the foregoing the relative percentages of our 50 cases in which these organisms occurred—Abel's bacillus 60%, Perez' bacillus 44%, B. proteus 40%—we find the results agreeing with those obtained by previous observers. Hence the presence of these bacilli in one condition and their absence in other conditions merits serious consideration. It is my opinion that the presence in ozena of Abel's bacillus indicates a pathologic relationship, and I would ascribe to it the rôle of an etiologic factor. When this bacillus has established a foothold in the nasal mucosa, the way is open for the superimposition of the ozena processes, which are dependent on the entrance of a definite species of bacteria.

CONCLUSIONS.

Since in the ozena stage of atrophic rhinitis Löwenberg-Abel's bacillus is found to predominate and since this organism is seldom present in healthy nasal mucosa, a relationship seems to be indicated between this bacillus and the pathologic condition.

Perez' bacillus can be isolated from an important percentage of cases of ozena. It is not present in normal cases or in infections other than ozena, and rarely exists in atrophic rhinitis.

The peculiar ozena odor is due to the presence of volatile products of protein-decomposition, belonging to the group of the organic sulfids, especially the mercaptans.

Such organic sulfids are produced by both Perez' bacillus and B. proteus, but not by Löwenberg-Abel's bacillus.

Perez' bacillus produces acute and chronic conditions in animals and it can be obtained from the nasal membranes of such animals.

B. bronchisepticus and B. proteus are pathogenic for rabbits and capable of producing the same clinical picture with its variations.

In view of the omission of proper controls, further work is necessary to warrant the full acceptance of Perez' bacillus as the most important etiologic factor in ozena.

THE THERAPEUTIC APPLICATION OF OVARIAN EXTRACT.*

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There is no more fertile, no more interesting field in research medicine than the domain of the internal secretions. The physiology of the endocrine glands covers the entire plane of internal medicine. The different members of the internal secretory system are closely linked to each other as well as to the other organs of the body. It would be interesting, no doubt, to trace out this close relationship that each gland with an internal secretion has to the other members of the endocrine family; but that is a subject in itself and time will not permit. The fact should be remembered, however, when any one member is up for discussion that hyper- or hypo-function of any gland, possessing an internal secretion, always has its effect upon the other members of the family.

The member of the endocrine group that will be discussed at this time is, as announced by the title of this paper, the ovary. It will be necessary, in order to bring out a rational therapeusis of ovarian extract, to review somewhat at length the anatomy and the physiology of the ovary. The ovary is a densely fibrous organ, situated in the pelvis on the posterior surface of the broad ligament in a shallow pouch—the "fossa ovarica." It is about 40 millimeters long, 20 millimeters wide and about 10 millimeters thick. It weighs about 5 gm. It might be stated here parenthetically that the ovary possesses two secretions, an external which is manifested by giving off of the ova, and an internal or the pouring into the blood of a hormone. It is the latter, the internal secretion, that forms the basis of this article. The ovary itself may be divided for our consideration into three parts, divisions that should be borne in mind during the later discussion on this subject. These three divisions are the follicle apparatus, the yellow body or corpus luteum and the so-called interstitial gland. Before taking up these separate divisions, mention might be made

*Read before the Kalamazoo Academy of Medicine, September, 1915, and before the Detroit Academy of Medicine, January, 1916.

of the enormous amount of literature on the subject of the glands with an internal secretion. Biedl in his book on the internal secretions gives the literature up to 1911 and has about 7,500 references. In his later edition he has added about 1,200 more. It will be seen, although not all the references are upon the ovary as a gland with an internal secretion, that an enormous amount of work has been done and is still being done upon the endocrine glands. But I am wandering far afield and must come back to the consideration of the different divisions of the ovary mentioned above. The ovarian follicle is perhaps so well known as to need no minute description, and it really does not enter into this discussion until after it has ruptured and expelled the ovum. The cavity left after this expulsion fills with blood and there is formed the so-called corpus hemorrhagieum. This blood clot organizes contracts and in its meshwork, cells are formed or enter from the membrana granulosa. By the disposition of the yellow coloring matter or lutein in these cells, the yellow body or corpus luteum is formed. This yellow body persists in the non-pregnant female until the next ovulation or about three weeks. In the pregnant woman, the corpus luteum verum usually last about five months, but otherwise differs in no way from the corpus luteum spuriousum or false corpus luteum. To repeat, lest there be some confusion arising from a misinterpretation of these terms, there is no difference, except perhaps of degree, between the corpus luteum of the menses, called the corpus luteum spuriousum, false corpus luteum or corpus luteum menstruonis and the corpus luteum of pregnancy, called the corpus luteum verum, true corpus luteum or corpus luteum gravidis. These terms are all used, and the thing to remember is that the difference is one of degree, not of kind. The interstitial gland or the third division is not so well understood, and much work is yet to be done before its accurate formation, origin and function are determined.

It is supposed that it arises from an incomplete primordial follicle; in other words from atresic follicles or follicles that have not reached their full and complete development. Bearing in mind this somewhat superficial description of these three divisions of the ovary, it is next obligatory that an explanation and application be made as to the function of each, in the female body economy. But at the same time it should be remembered that

these three divisions—*i.e.*, the follicular apparatus, the corpus luteum and the interstitial gland—do not have their functions sharply defined, the one from the other, and at times they all work together in perfect harmony in carrying out the internal secretory power of the ovary.

Some one has said *"Mulier propter ovarium est, quod est."* It is the consensus of opinion now that it is not a nervous influence but chemical substances—hormones, if you will—which cause the deep changes in the female organism. This fact has been proved by the results of ovarian transplantation. But whether the ovarian hormone is produced in the follicle in the corpus luteum or in the interstitial gland is at present uncertain. But certain it is that the internal secretion of the ovaries brings about a protective influence upon the development of the sexual peculiarities of women. In fact it must be admitted that even in the prepuberital period of development in young girls, characteristic body and psychical signs are present, which differentiate in a marked way the female sex from individuals of the male sex of the same age. Further that the ovary possesses an internal secretion even before puberty. For these phases the follicular apparatus and the interstitial gland can only be considered. But with puberty there comes a change in the cyclical life of the female, which is shown objectically by the appearances of the menses. The most important cause of the cyclical appearing menses is to be found in the ovary, for by its removal at operation, by its atrophy at the menopause or by its destruction by disease, the menses stop. In the ovary, at regular intervals two morphologic and periodic changes occur, the ripening of the follicle and the formation of the corpus luteum. According to the investigations of Leopold and his associates, the idea was prevalent that all processes, at the time of the menses, come with the bursting of the follicles, but this has been modified somewhat. Fraenkel, after a series of histological and clinical investigations, came to the conclusion that the menses are ushered in, not by the ripening follicle, *i.e.*, ovulation, but by the corpus luteum, and that the menstruation begins at that moment in which the yellow body has reached its full development. Exact histological studies upon the different phases of the development of the corpus luteum by several German research workers have proved that, not as was

formerly supposed, the menses started with the bursting of the ripened follicle, *i.e.,* menstruation and ovulation occur synchronously, but that ovulation occurs in the interval, ca. ten days before the menses are ushered in. Then a corpus luteum is formed in the cavity of the ruptured follicle, which yellow body, when it has reached its full development, ushers in the menses by means of a hormone that it, the corpus luteum, secretes. So that the former statement, without ovulation, no menses, must be modified to read, without ovulation, no corpus luteum formation; without a corpus luteum, no menses.

Besides this power of causing the appearance of the menses, the corpus luteum has another power, that is to promote the ripening of the ovum and to prepare the uterine mucosa for the reception of the fertilized ovum. To speak for the moment chronologically this is what happens every month during the life of the female from puberty to the menopause. A follicle ripens and at its full development bursts and discharges the ovum into the abdominal cavity. This is ovulation. In the cavity of the follicle a corpus luteum is formed that at its full development ushers in the menses. Then the corpus luteum begins to degenerate even before the menses stop. Certainly by the end of the second week the corpus luteum will have taken a hyaline appearance and the so-called corpus ablicans is formed. Soon or in a few days another follicle starts to ripen and the above process is repeated.

If the discharged ovum become fertilized, then the corpus luteum formed does not usher in the menses, but prepares the uterine mucosa for the reception of the fertilized ovum and aids in the formation of the decidua and placenta. This has been most admirably demonstrated by the researches of Leo Loeb. He could show that the development of the decidua is dependent upon the presence and function of the corpus luteum. He was successful, in guinea pigs and rabbits, by external stimuli, such as scarification of the uterine mucosa, by the placing of a glass rod in the uterus, etc., in producing a formation of an artificial decidua, providing a yellow body was present in the ovary. Loeb explained the mechanism in this manner, that a hormone is produced in the corpus luteum which renders the uterus sensitive. The premenstrual swelling of the mucosa, which histologically

bears a great resemblance to the true decidua, takes place through the action of the false corpus luteum or corpus luteum spuriousum. With cessation of the function of the corpus luteum, and the entrance of the menses, the beginning decidua retrogrades and disappears. If the ovum is fertilized, then the corpus luteum remains longer in action and the decidua develops and later the placenta.

The function of the corpus luteum verum or true corpus luteum does not continue through the entire pregnancy. According to the careful and painstaking investigations of Seitz, the atrophy and retrogressive change of the corpus luteum of pregnancy begins slowly, probably about the middle of the third or beginning of the fourth month. In the later months, the degeneration increases to such a degree from distention and enlargement, that at the end of pregnancy no trace of the former yellow body can be made out except perhaps the resulting hyaline scar.

After the fourth month of pregnancy, when the corpus luteum ceases to functionate, it is supposed that its work is taken up by the interstitial gland which has no doubt been formed to take up the work of the corpus luteum, even before the latter has ceased to functionate. The interstitial gland of the ovary is supposed, according to the two French investigators, Bouin and Limon, who first discovered and described this gland, to spring from atresic degenerating follicles. It is according to some not developed in the same way in all animals nor at the same time in the same animal, is its period of development the same. It is, however, agreed by all that the most complete development is during the latter months of pregnancy. The small, faint, thin spindle-shaped cells of the theca interna of an atresic follicle becomes thicker, larger, more voluminous, oval or pointed; their protoplasm contains many fat droplets and often shows a light yellow color. From the lutein-like characteristics, they are called theca-lutein cells. These cells, arranged radically and longitudinally in ornate groups, surround the still preserved follicle space with a broad band of cells containing fat. It has also been observed that the older the pregnancy, the better developed is the interstitial gland. This gland is well developed in the hydatid mole, which as is well known arises from a proliferation of the syncytial and of the Langshans cells. It is also found in the chorio-epithelioma.

Seitz, who has reported at length on the internal secretion of the ovary, in a monograph read before the 15th German Gynecological Congress upon the relation of all the endocrine glands to pregnancy, comes to the following conclusions, as regards the ovary:

1. The internal secretion of the ovary plays an important part in determining the sexual characteristics of the female.

2. The corpus luteum ushers in the menses and prepares the uterine mucosa for the reception of the fertilized ovum.

3. It is important to note whether, in women who are prone to abortion, the fault does not lie in imperfect development of the corpus luteum.

4. The function of the corpus luteum comes to end in the first third of the pregnancy.

5. In the later months of pregnancy there develops an organ called the interstitial gland which takes up the work of the corpus luteum.

It must be remembered in this somewhat hasty review of the anatomy and physiology of the internal secretory apparatus of the ovary, that no attempt has been made to treat the subject exhaustively. The desire is to simply pave the way for the more practical part of the paper, as indicated by its title.

While many investigators have been much occupied with the physiology of the ovary as an internal secretory organ, almost all have, more or less been working to obtain the active principle, as has been done in some of the other endocrine glands, *e.g.,* the adrenals. This question has been attacked in many ways with about as many varying results. Up to most recent times, the ovary has failed to yield the active principle of its internal secretion. The writer has been more or less active in this work for the last eight years and only recently has he met with any success. In fact up to the present time practically no results have been obtained therapeutically, except with the desiccated products. The first step was to prepare the whole ovary by careful handling and administer it as a dried product. Next the corpora lutea were separated and dried at body temperature and given in capsules of 5 grains, one-half to one hour before meals. During the last year a water-soluble powder was obtained from the corpus luteum, and as far as we have been able to judge, this will be a

great advance in the determination of the chemical contents of the active principle of the ovary or the corpus luteum.

The long and laborious processes by which this is accomplished will not be discussed here, as most of you are more anxious to hear the indications for its use in every-day practical medicine.

The most common condition in which some form of ovarian material may be used, is the troublesome symptoms of the menopause, either artificial or natural. The usual complaints are flashes of heat or cold, insomnia, nervousness, etc. It is surprising what alleviation of these troublesome and unpleasant disorders can be accomplished by the use of desiccated corpus luteum, properly administered. A report of research cases will be found in another paper, written some years ago. One great advantage in this medication is that it does not do any harm if no relief is accomplished. As far as can be learned from the use of soluble product, relief may be obtained in less time with fewer doses, and a relief that will be more permanent than with the dried gland. Perhaps the action of the gastric juices has some effect upon the efficiency of the product. At all events it is always better to use the active principle, where it can be obtained, than to trust to the action of the entire gland with its varying amount of inert substances.

From what was said above about the function of the corpus luteum being able to usher in the menses, the use of either the desiccated product or the soluble extract is often productive of good results in amenorrhea or in scanty menstruation. Seitz working with the corpora lutea of beef ovaries was able to isolate two antagonistic bodies which he called luteo-lipoid and lipamin. The former was found, by animal experiment and clinical observations, to possess blood inhibiting characteristics, and subcutaneously injected before and during the menses to lessen the flow and shorten it. The second body, lipamin, a lipoproteid and a lecithin albumin, causes an increased growth of the external and internal genitalia in animals. In women, by subcutaneous injection, it will cause the menses to appear in amenorrhea. If the results of Seitz are correct as regards these two antagonistic bodies found side by side, so to speak, in the corpus luteum, it is no wonder that many times no clinical results are obtained when the entire gland is administered. Seitz explains the presence of

these two bodies, possessing this antagonistic action in this way. Both these substances, luteo-lipoid and lipamin, are present in the corpus luteum, but at different times and in different amounts. In the young corpus luteum, the lipamin has the upper hand and the flow appears. Later the luteo-lipoid is present in larger amounts in the corpus luteum and the menses stop. If this assumption is correct, then it must follow that in the young corpus luteum there is more lipamin and in the older gland more luteo-lipoid. This was proved by Seitz and his co-workers by histo-chemical and by quantitative chemical methods. Hence in order that the menses may appear regularly, be of proper duration, and that the loss of blood may be of the proper amount, it is necessary that the time and quantity balance of these two bodies be correct. All this goes to show that until the active principle or active principles, if you will, of the internal secretion of the ovary are isolated beyond peradventure of a doubt, its therapeutic use will be more or less problematical.

Before summing up the therapeutic possibilities of the ovarian secretion, a moment will be taken to give you an abstract of case that occurred at the Maine Agricultural Experiment Station at Orono, Maine. This case was reported by Pearl and Surface under the title "The assumption of male secondary characteristics by a cow with cystic degeneration of the ovaries." A pure-bred Ayrshire cow had produced three calves in September, 1909, in September, 1910, and February, 1912. After March, 1913, the cow never gave any milk. The udder rapidly shrunk to a very small size and the animal began to show the external characteristics of a bull. After a lapse of eight months the general external facies and the behavior of the cow were like those of a bull to a remarkable degree. If the cow had been so screened that only her fore-quarters and neck were visible, any observer would have pronounced her a male, without question. The cow was killed February 18, 1914. Autopsy showed as the only gross abnormality a simple cystic condition of the ovaries. But, and here is a most important point, histo- and cytologically these cystic ovaries differed from the normal cow's ovary in but one essential point, namely, that they had no corpora lutea. In summing up this case, the reporters present the following:

1. This cow had been a perfectly normal female and had per-

formed all the reproductive functions, both primary and secondary of the sex.

2. It later assumed certain of the secondary characteristics of the male, both in respect of structure and behavior with perfect definiteness, and so far as the character concerned goes, completeness. The change was like that following the castration and transplantation of gonads.

3. The gonads of this animal, examined subsequently, were exactly like those of a normal cow, save in one respect, that the follicles were not breaking and discharging ova, but were forming follicular cysts or becoming atresic, and because of this no corpora lutea were formed.

This case shows that in the animal a lack of corpora lutea formation causes the female to assume the characteristics of the male. Perhaps here is another possibility for the therapeutic application of some form of the ovarian secretion in the human body.

In conclusion, perhaps it will be only necessary to mention some of the possibilities of the active principles of the ovary, when it is isolated in a pure form. Certain it is that the ingestion of the dried gland per ora is not without its objections. For can we be certain that, in whatever form given, the gastric juice does not alter or render it inert. So that, whether we try to alleviate the troublesome and unpleasant symptoms of the artificial or natural menopause, whether to cause the menses to reappear in amenorrhea, we must be sure that we are giving the internal secretion of the ovary in its proper form, at the proper time, and in the proper amount.

The question of infantile genitalia, of hyperemesis, of the correction of abortion, may be helped, bettered or even cured by treatment with ovarian secretion, but this form must be worked out before any accurate data can be given. Also the question of interdependence or interrelation of the different endocrine glands must be studied in order to combat overfunction or hyperfunction of any of its members. It is comparatively easy to prescribe for a lessened or hypofunction of the ovary, for example, but suppose for instance that the ovary is producing its internal secretion in too large amounts. It would, of course, be suicidal to give ovarian extract in any form. It would only aggravate the con-

dition present. This is only one of the many problems that confront the research worker who is busy with the internal secretions of the different members of the endocrine series.

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EXPERIMENTAL SYPHILIS.*

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The discovery of the *Treponema pallidum* by F. Schaudinn (1) brought the study of syphilis within the scope of medical research. Because of the lack of definite data concerning the nature, morphology and distribution of the causative organism, our conception of this disease, founded almost entirely upon clinical observation, had been very fragmentary, until the time of Schaudinn's discovery. It is the purpose of this paper to review the results which the work in this field of research has yielded, and to point out the application of the experimentally elicited knowledge to the accepted facts and to some of the problems which still present themselves.

Inasmuch as numerous organisms had been described as the etiologic factors in this disease, the announcement that the pallida was the causative agent of syphilis met with considerable skepticism. It became necessary, therefore, to establish the causal relationship of the *Treponema pallidum* to syphilis. This was accomplished by:

1. The observation of the organism in the syphilitic lesions incident to the various stages of the disease.
2. The successful inoculation of lower animals from human lesions, thereby producing syphilis experimentally in rabbits, monkeys and other animals.
3. The growing of the *Treponema pallidum* in culture media free from contamination, the transfer of these cultures through many generations, and the successful inoculation of lower animals with the cultivated organisms.

Sufficient evidence has been brought forward in recent years to show that the *Treponema pallidum* occurs regularly in all stages of syphilis. In the systematic search carried out during the past eight to ten years, the presence of this organism has been demonstrated, in the early syphilitic lesions, which from clinical observation must be considered infectious. Likewise, in the later stages of this disease, the pallida has been found to be the cause of the degenerative changes observed in the cardio-vascular system and connective tissue (2). Noguchi and Moore (3) succeeded in demonstrating the pallida in the brains of general

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paretics and in the lesions of the spinal cord of tabetics. The noticeable number of syphilitic reinfections described in the literature during the early period of the salvarsan era led to the examination of the scar tissue of healed primary lesions. Microscopic examination disclosed the fact that such scar tissue may contain the pallida. Conclusive evidence was furnished by the experiments of Hoffman and Sandmann (4), who by inoculations with the scar tissue of healed primary lesions succeeded in transmitting the disease to monkeys. These foci of organisms may persist for considerable periods, giving an indication of the length of time the pallida may persist in a state of quiescence.

That the presence of the pallida need not be coincident with manifestly syphilitic lesions was pointed out by Campbell (5). The occurrence of the pallida on the unaffected surfaces of tonsils in secondary and latent syphilitics may be just previous to or shortly after the appearance of a lesion in the mouth. Once the infection, as manifested by the primary lesion, is well established, the pallida invades the lymphatic system and only subsequently makes its appearance in the blood. Attempts to cultivate the pallida directly from the blood have been unsuccessful. The organism does not seem to grow well in the presence of blood. Inoculation experiments were carried out by us on rabbits, by injecting immediately after it was drawn five c.cm. of blood from patients exhibiting a well marked secondary eruption into the ear vein of the animal. These experiments proved successful in three out of five attempts.

If, however, the blood was allowed to coagulate, the inoculations were negative. It is possible that the pallida are so firmly held by the fibrin in the clot that the grinding of the clot and the subsequent filtration to get rid of the coarser particles also remove most of the organisms. The lesions appeared in the testes of the successfully inoculated rabbits about four to six weeks after inoculation. In no other organ or gland could the pallida be found, although we examined fresh preparations from them under the dark-field. Levaditi and Petresco (6) demonstrated the pallida in the serum of blisters resulting from the application of cantharides plasters. C. H. Hermann (7) made similar observations.

The search for the *Treponema pallidum* in the cerebro-spinal fluid yielded negative results at first; later the organisms were found and described by Rosenberger (8), also Sazary and Paikard (9). That the urinary sediment in syphilitic nephritis may con-

tain the pallida has been shown by Hirshberg (10) and McLennan (11).

Long before the organisms had been observed in microscopic preparations from tertiary lesions, the presence of the pallida in these lesions was demonstrated through animal-inoculation. The early negative findings led Schaudinn to the assumption that the pallida might be present in a changed form, representing possibly a resting phase in the life cycle of the organism. Through painstaking and persistent search, Doutrelepont and Grouven (12) and Tomasezewski (13) succeeded in finding typical forms of the pallida in gummas and other tertiary lesions. Thus, the experimental work was corroborated by their pathological findings. Throughout the entire course of the disease the pallida may be demonstrated in the lesion, characteristic of the successive stages of acquired syphilis.

Congenital syphilis apparently presents no exception. In acquired syphilis the fact has been noted that the pallida may remain alive in the organism over extended periods of time without giving rise to recognizable syphilitic lesions, or even to symptoms which might lead us to suspect their presence. It is fair to assume that congenital syphilis may likewise exhibit similar periods of latency, noted in children of syphilitic parentage, who may not present any of the signs and symptoms of the disease, during their first years. That the pallida is the cause of subsequent syphilitic manifestations is shown by the findings of Hoffmann, who demonstrated the pallida in the blood of a congenital syphilitic child; and again by Igersheimer (14), who found the organisms in the cornea of a congenital syphilitic boy of fourteen.

Congenital syphilis has offered exceptional advantages for the study of the influence which the presence of the *Treponema pallidum* exerts on the vascular system and adjacent tissues. Almost all organs and tissues may harbor the pallida. It may be found in varying numbers in sections prepared from the liver, spleen, kidneys, suprarenals, muscle and heart muscle; also in the bone marrow and in the epiphyseal zone of the long bones, in the blood and skin, and in inflammatory areas of the meninges. The occurrence of the pallida in the mucous membranes of the stomach and intestines has been pointed out by E. Fraenkel (15) and Simmonds (16). The last named author found them also in the brain, spinal cord, ovaries, uterus, testes, prostate, and bladder. The pallida are not found evenly distributed throughout the body, nor in all the organs. Their distribution seems to depend on the severity of the infection, which may be septicemic.

More often the syphilitic lesions are restricted to several regions or organs. Stillborn fetuses show a greater degree of infection than children who succumb to the disease after birth. The liver, stomach, and intestine seem to be the organs first invaded; next in order are the lungs, suprarenal glands, and the skin. The invariable occurrence of these organisms in the liver is due to the fact, as has been pointed out by Levaditi (17), Gierke (18) and Beitzke (19), that the pallida are carried from the syphilitic mother through the umbilical vein to this organ. Lymph glands, spleen and thymus are less frequently affected.

The distribution of the *Treponema pallidum* corresponds in a general way to the visible pathological changes. The pallida, however, may occur in regions where no changes from the normal are noticeable, and may be entirely absent in regions which have undergone extensive tissue changes. The pallida will then be found in the tissues surrounding such lesions. The slow and protracted toxic action of the pallida, no doubt, accounts for its occurrence in localities exhibiting normal histological conditions before pathologic tissue changes have had time to develop; and may also be the reason for their absence in areas which show pronounced degenerations, characteristic of the disease.

The walls of the blood and lymph vessels, the interstitial tissue and capsules of the organs frequently exhibit the organisms in very large numbers. One may also find them free in the lumen of the vessels. The parenchyma of the organs is invaded later, and here they occur mostly intercellular. Gierke (20) and Frohwein (21) have observed them intracellular. It is still an open question whether the appearance of the pallida within polynuclear leucocytes is due to phagocytosis, or whether, as appears to be in case of the parenchyma cells of the liver and salivary glands, the organisms migrated into these cells. The nature and extent of the defensive cells' reactions of the body against the infection of the *Treponema pallidum* is as yet little understood. The occurrence of the organism in relatively small numbers in the spleen and lymph glands has led to the assumption that the pallida are destroyed in those organs. Systematic examinations of placental tissue have disclosed the presence of the pallida, either in the maternal or in the fetal part of the organ, or both. That the treponema does not occur with any frequency in the placenta has been pointed out by Wallish and Levaditi (22), who found the *Treponema pallidum* in only one out of thirteen placentas of syphilitic women. Mohn (23, 24) found them in seven cases out

of fifteen. The placenta will be found to contain the pallida whenever the fetus shows signs of the infection. Therefore, it seems probable that the pallida reaches the placenta from the fetus, which has been infected from the mother during the early period of gestation. Trinchese (25) expresses the view that the embryo is infected through the pallida in the maternal blood, and not through the infection of the ovum as has been considered possible by Mohn. Trinchese's view is furthermore supported by the observation of Fieux and Mauriac (26), who noted the birth of a syphilitic fetus from a case of post-conceptional infection.

These observations, as well as the numerous communications which have appeared, leave little doubt that the *Treponema pallidum* is almost invariably present in all forms of syphilis. Their demonstration is of great diagnostic value in clearing up doubtful cases and in leading to the application of specific therapy. Because it is easier and more definite, the diagnosis by means of the stained preparation or the dark-field is in many cases superior to the serodiagnosis of Wassermann.

There were in earlier times a few attempts at inoculation in human beings, but these sporadic efforts could never serve as a basis for systematic experiments. A satisfactory solution of the conditions necessary for infection and immunity could be obtained only through animal experiments. The transmission of syphilis to apes, monkeys and rabbits by inoculation must for this reason be considered a decided step in advance. Metchnikoff and Roux (27) were the first to succeed in the inoculation of apes. The possibility of transmitting the disease to rabbits was first demonstrated by Bertarelli (28, 29), who also successfully inoculated dogs and guinea-pigs. Uhlenhuth and Mulzer (30-38), Truffi (39), Neisser (40, 41), took up this work for biological, immuniological and therapeutic studies. Noguchi (42) and myself (43) used the testicular lesions of rabbits for cultivation experiments.

To inoculate the lower animals successfully, it is necessary that the material employed should contain the pallida in a viable form. It should be fresh and sufficient in quantity. It is best to have the animals at hand and to carry out the operation as soon as the material is obtained. The material should not be divided into very small pieces for the sake of inoculating a large number of animals. The chances of success are better if fewer animals are used, and the inoculation dose increased.

Apes and monkeys are inoculated by the cutaneous method.

The skin is scarified fairly deeply and the syphilitic tissue is rubbed thoroughly into the scarified area. The uninjured skin does not permit the passage of the pallida, for all attempts at inoculation without previous scarification have been negative. The inoculation may be made on any part of the body of higher apes, though the forehead is usually the site selected. Monkeys and rabbits may be inoculated into the testis. With rabbits the inoculation into the anterior chamber of the eye is also a common mode of procedure. Such experimentally produced lesions may serve as inoculation material for further transplantations. If the tissue used for inoculation has been free from contamination, the wound produced through scarification will heal completely. After a period of incubation of from fifteen to sixty days the primary lesion will appear, in the form of one or several papules. These papules coalesce and give rise to a typical primary ulcer, surrounded by an indurated area, which may persist from several weeks to a month and then heal. Not every successful inoculation will give rise to secondary lesions. Such secondary eruptions have been observed on apes by Metchnikoff and Roux. Inguinal adenitis frequently follows inoculations into the testis, yet the pallida do not occur in large numbers in these glands. Aside from the presence of the pallida in the lymphatic glands draining the infected area, Neisser has demonstrated the organisms in the spleen and bone marrow of infected animals.

Bertarelli (28, 29) was the first to demonstrate that rabbits could be inoculated with syphilis. Through the injection of finely ground syphilitic tissue into the anterior chamber of the eye of rabbits, he observed specific changes in the cornea. He likewise demonstrated the organisms in the lesions, both under the microscope and through further transplantation from rabbit to rabbit. Attempts by this author to produce syphilitic infection through subdural inoculation gave negative results. Inoculation into the skin gave only occasionally positive results.

The discovery that the testes and the coats of these glands were especially susceptible to the syphilitic virus, was of great importance in the field of experimental syphilis. The treponema may be passed from rabbit to rabbit, thus yielding material very rich in organisms, and, in the course of transplantation, may become free from all contamination. Depending upon the technique of inoculation, the lesions produced by these transplantations vary from a typical chancre to a gumma. If the infected tissue is placed directly under the skin of the scrotum, a chancre

will develop. If the inoculation material is inserted between the layers of the tunica, thickening of these layers will occur, due to the increase of connective tissue. The inoculation of the gland itself is followed by inflammatory reaction and swelling which after their subsidence result in mucoid degenerations of parts of the glands. These areas may be detected by palpation. On dissection, they appear pearly white, stringy, and gelatinous. Such areas are very rich in the *Treponema pallidum*. The period of incubation varies from ten days to two months. The lesions will heal spontaneously. Opinions differ as to whether such animals have acquired an immunity. In my own series of transplantations, fourteen rabbits which had developed gummas subsequent to the first inoculation, did not contract syphilis from a second inoculation, irrespective of the strain used. Following the third reinoculation, one rabbit developed lesions. These negative results may be due to errors in technique, or the animals may have still harbored the pallida from the previous inoculation. There was no apparent lesion, however, which might have suggested this.

During the past three years, we have inoculated about eight hundred rabbits with an average take of 25 per cent. The number of successful inoculations depends on the virulence of the organisms, the resistance of the animal, and on the circumstances attending the time of operation. The *Treponema pallidum* is easily injured. Strains may be kept for long periods by successive transplantations in rabbits. The results of our experiments show that we have carried four strains in this manner for about two years. The pallida do not lose their pathogenicity by passage through rabbits; for a case of accidental inoculation in the finger while performing transplantation is reported, which was followed by a characteristic primary lesion at the site of infection.

In a few cases generalized syphilis in rabbits following inoculation has been observed. Uhlenhuth and Mulzer (37, 38) noted generalized syphilis in rabbits and monkeys following intravenous inoculation. Grouven (44) was able to observe secondary lesions within two years in monkeys, and within one year after inoculation in rabbits. Sowade (45) observed it in rabbits following intravenous injection of the culture of the pallida. Baermann (46) describes the occurrence of secondary lesions following the subcutaneous inoculation in monkeys. We have never seen a case of generalized syphilis in our series of animal inoculations, not even in those animals injected into the ear vein with large quantities of blood from secondary syphilitics. Of especial inter-

est in this connection are the observations of Arzt and Kerl (47), who describe the occurrence of a spirochæte, possibly saprophytic, in non-syphilitic lesions on the genitalia of rabbits. The organisms in question closely resemble the pallida morphologically. The lesions in which they were observed were flat ulcerations. In one instance they were also found in a papule in the mouth. In four cases the organisms could be demonstrated in the regional lymph glands. The infection could be transferred from rabbit to rabbit, though not to monkeys. The authors examined in all eight hundred and fifty animals from various sources, of which seventy-two had the above mentioned infection. Although the possibility of a generalized syphilitic infection in rabbits following inoculation must be conceded, especially when the injection has been made into the blood stream directly, and with large quantities of material, the instances cited are exceptional.

Experimental syphilis permitted comparative histological and pathological studies of the lesions produced by the pallida in man and the lower animals. It also served as a means for obtaining material very rich in organisms, and this material was the starting point for the attempts to cultivate the organism *in vitro*.

In cultivating the *Treponema pallidum* the methods employed in this country and abroad differ, in that investigators in this country used the experimentally produced lesions in rabbits to cultivate the pallida, whereas the European investigators made use of the tissue from human syphilitic lesions directly. The pallida was grown in pure culture first by Noguchi (48, 49), who used serum water and normal rabbit's tissue to grow the organism. The cultures were kept under hydrogen. The workers abroad—Mühlens (50, 51), Hoffman (52, 53), Schereschewsky (54), Sowade (55), Tomasczewski (56, 57), and others—made use of partly coagulated horse serum for its cultivation. The claim to have succeeded first in growing the pallida in pure form is made by Mühlens, who in describing his cultures called especial attention to the characteristic pungent odor produced by the pallida. That the odor was due to contaminating organisms there is little doubt, for the cultures of Noguchi as well as our own are devoid of any odor. Nor could it have been due to the horse serum employed, for we have cultivated the pallida according to the methods described by Noguchi and those made use of by Mühlens without noticing any odor.

The pallida grows diffusely in solid culture media, giving it a hazy appearance. The growth extends to within one-half inch

of the top of the agar column. In liquid media the growth extends in the form of a faint cloud to about the same height. In the older cultures, the organisms settle as a fine flocculent precipitate to the bottom. The organisms grown in liquid media exhibit characteristic motility. Those grown in solid media are less motile.

The pallida when carried in culture will soon lose its pathogenicity for rabbits. The injection of large amounts of emulsified ascitic agar cultures has never resulted in a lesion. There are no data available as to the pathogenicity of the pallida in culture toward man.

The greatest difficulty, therefore, in attempting to cultivate the pallida consists in obtaining the first take on inoculation of culture media. Not all strains of this organism lend themselves to cultivation, or to inoculation experiments. Even after the pallida have begun to grow, the process of eliminating the contaminating organisms calls for perseverance and patience. The transfer of the cultures once pure, simply calls for the necessary precautions to prevent accidental contamination. The cultures are transferred once a week. The pallida will grow, however, in deep ascitic agar for several months.

Observed under the dark-field, the different strains under cultivation when compared with the organisms taken directly from the human lesion vary in thickness and appearance. This variation may be due to the influence of the culture media employed. Very short as well as very long forms may be observed in the same culture. At times, cultures are found in the same strains in which the organisms exhibit fine, filamentous processes at each end. These processes are so fine that it requires ideal conditions to observe them. Wide variations occur also in respect to the number, width, and length of the spirals. Individuals may be observed which are partly straight, exhibiting the spiral form on one pole only. There is also some variation in thickness in the organisms in the same culture. Aside from these peculiarities some strains of the pallida are thin, others thicker. The characteristics of the individual strains are better preserved in liquid than in solid media. Whether the thicker forms possess an affinity for the nervous system as indicated by Nichols (58), Noguchi (59), Uhlenhuth and Mulzer (60), and Wile (61), has not been definitely established.

The pallida multiplies by transverse division, for one can find in cultures forms exhibiting a delicate plasma bridge between

two individuals. Longitudinal division of the pallida has been described by Herzheimer (62) and Loewy (63); Nakano (64) in describing his observations on the division of the pallida expresses the opinion that the longitudinal divisions observed were simply the free ends of two individuals wound around each other. The technical difficulties involved in successfully staining the *Treponema pallidum* have hampered the study of the cytology of the organism. Noguchi describes the presence of small granules in the protoplasm of the organisms which are set free. Whether these granules are in any way connected with reproduction is still open to question.

It was formerly supposed that syphilis is a disease to which man only is susceptible. The experiments of Metchnikoff and Roux (27) in 1903 were the first positive indication of the possibility of experimental syphilis in apes. Since that time our conceptions regarding the natural immunity of animals have undergone a change. With the ability to transmit syphilis to lower animals and the cultivation of the pallida in pure form, attempts at immunization were undertaken. Nakano (65), who treated rabbits with killed cultures of pallida, could detect agglutinins in their blood, while precipitins were absent. He also believed that he noted specific complement fixation. With Pfeiffer's experiment he was able to detect a weak lysin. Active immunization experiments with cultures of the pallida were negative, as were also his attempts to immunize human beings with a vaccine prepared from such cultures. He found that the serum of immunized rabbits possessed neither immunizing nor therapeutic properties. The pallida in the tissue exhibits a high resistance to bactericidal substances. Nakano found living pallida in primary lesions after injecting them with 10 per cent. antiformin solution. Kolmer (66), who also immunized rabbits with cultures of the pallida, described the presence of complement-binding antibodies and agglutinins. He was unable, however, to demonstrate agglutinins in the serum of syphilitic patients, even when they gave a positive Wassermann reaction. Schereschewsky (67), who likewise carried out immunization experiments, prepared the vaccine by means of antiformin solution and heat. By injecting the vaccine intravenously, he was able to immunize two monkeys. He noted the presence of precipitins as well as complement-binding antibodies in the serum of rabbits treated with the vaccine. He, therefore, concluded that the immune bodies which he was able to produce and demonstrate were the result of the endotoxins of the pallida, liberated through the antiformin treatment.

Zinsser, Hopkins, and Gardner (68) also immunized rabbits with cultures of the pallida, and were able to note the presence of agglutinins. The sera of rabbits immunized with one strain agglutinated another strain in a dilution of 1:500. They also made the observation that the normal human sera as well as sera from certain syphilitic patients with positive Wassermann reaction would agglutinate similar pallidum emulsions. The dilutions of the sera of syphilitics and non-syphilitics which caused agglutination of the suspensions of the pallida are not given, so that it is impossible to determine whether the difference between the two types of sera is sufficient to be of diagnostic value. It is evident that immunity against the pallida, as far as it exists, is not absolute. The experiments on record to date are not sufficient to determine this question.

Although the observations of generalized syphilis in the lower animals following inoculation are comparatively rare, the clinical manifestations, as far as they occur, do not differ materially from those observable in man, who must be considered the most susceptible to this disease. Syphilis has never occurred in an endemic form among healthy and inoculated animals, when kept together. There is in man no hereditary immunity to syphilis. The observations that certain localities and races are entirely or relatively free from syphilis is due, not to immunity to this disease, but to social restriction and religious customs.

An individual disposition which may result in a severe or light course of this disease may be assumed. Experimental work has yet afforded us no indication that malignant syphilis is due to a special type of organism. The occurrence of the severe types of syphilis in cases of tuberculosis and in individuals weakened by other diseases, as well as the observation that individuals infected from those having malignant syphilis exhibit the disease in a milder form, or *vice versa,* indicates that the factors determining the course and severity of the disease are either a weakened condition of the individual, or an individual idiosyncrasy toward the *Treponema pallidum.* The frequency or entire absence of tabes and general paresis may be due to this individual disposition and to the effect of alcohol, which no doubt renders the nervous system of the individual more susceptible to the infecting organisms. This predisposition may also be racial.

By acquired immunity we understand the temporary or permanent ability of an individual to overcome subsequent infection after having recovered from the first attack of the disease. The

defensive forces of the body which formed during the first attack make it impossible for subsequent attacks to develop. The dictum of Ricord that no one could be subject to constitutional syphilis twice, that is, that there existed an acquired resistance to a second attack of syphilis, cannot be maintained, inasmuch as reinfections with the *Treponema pallidum* after complete recovery from the first infection have been recorded. E. Klausner (69) describes such a case, which, on account of the carefully controlled observation, is of interest. The patient, who presented himself with the initial lesion (spirocheta positive, Wassermann reaction ++ positive), lymphadenitis, and a papulo-pustular exanthem, was treated with salvarsan and discharged after the symptoms had disappeared and the Wassermann reaction had become negative. One year later this patient exhibited a new initial lesion (spirocheta positive), Wassermann reaction negative, regional and universal lymphadenitis, and a universal maculo-papular exanthem. With the appearance of the exanthem, the Wassermann reaction became again positive. In reviewing the literature on this subject, it becomes evident that not all cases of reinfection described are such, as single secondary lesions have been observed. These pseudo-reinfections are due to the liberation of the pallida from syphilitic foci which, not influenced by the therapeutic measures taken, spread as soon as the condition of the patient is favorable for it. In a sense this also is reinfection, but with the same strain of organisms. Krefting (70) cites a number of cases of reinfection, and describes the recurrence of the primary lesion at the site of the old, two months later.

Klausner suggests the following criteria to distinguish between a pseudo-reinfection and a reinfection in the sense that the disease was cured and the patient contracted syphilis a second time: An unquestionable history of the first infection, the diagnosis of a second infection, based on the initial lesion, typical changes in the lymph glands and appearance of the secondary symptoms. Of great importance, though not absolutely necessary, is the Wassermann reaction, negative in the beginning, and becoming positive as the secondary exanthem manifests itself. John (71), who reviewed the literature on this subject, collected three hundred and fifty-six cases of reinfection, and of this number only one hundred and nineteen could be classed as true reinfections. Hutchinson, J. (72), describes seven cases of reinfection which came under his own observation. He points out, in conclusion, that the interval between two attacks of syphilis

may be so short a time as eighteen months, *i.e.*, a patient may no sooner have finished his course of treatment than fresh exposure may produce a complete fresh attack. The average interval between the first and second attack, in the seven cases cited by him, was six years. In the same communication he calls attention to eighteen cases of undoubted reinfection observed by his father. Of special interest is the observation of an existing double infection in a hereditary syphilitic.

Since the advent of the salvarsan therapy, reports of complete cures have increased. Such cases have in a number of instances contracted syphilis a second time, exhibiting it at times in a milder, at times in a more severe, form than during the first attack. Although these observations are scattered, they demonstrate that no immunity is produced by reason of having overcome this disease.

Neisser and Finger have pointed out that no immunity existed against the virus causing the first infection, since syphilitic lesions made their appearance years after the primary infection. The conception of immunity plus reinfection in syphilis appears inconsistent in view of the fact that the recurrence of syphilitic manifestations in an infected individual is a common observation. Nor can we readily assume the presence of an immunity against a subsequent infection of the pallidum, since we observe none against the one giving rise to the first attack. Clinical experience as well as experimental data have established the fact that the appearance of the primary lesion renders the superficial layers of the skin non-susceptible to a new infection. This may be due to a regional immunity, which gradually extends and is fully developed during the periods of latency; or it may be due to a non-susceptibility to foreign syphilitic virus, while the susceptibility for the virus causing the infection remains. This non-susceptibility of the skin to the virus is developed gradually. It is a fairly frequent occurrence to observe two or more indurated primary lesions situated on parts of the body which are not directly continuous, but which touch each other, as, for example, the penis and scrotum, the labia majora and minora or the upper and lower lips. These are probably examples of autoinoculation, although the possibility of a simultaneous infection in two places must be conceded.

Finger and Landsteiner conclude: "Reinoculation is successful in proportion to its proximity, in point of time, to the primary inoculation. If general infection is not yet complete, a typical

chancre can be produced, but from the time when constitutional symptoms appear, it becomes more difficult to succeed. During the secondary period the result has some semblance to a secondary papule." F. J. Lambkin (73) says of the primary chancre: "It is not readily autoinoculable and only so during the first ten days of its existence." He found the possibility of autoinoculation from the penis on soldiers within ten days of the existence of the primary sore. "After ten days I found it impossible to reproduce an identical sore or anything like it. As a rule, a small inflammatory pustule was the only result. Hutchinson describes three cases of autoinoculation before the secondary symptoms developed, and one case where an injury on the right index finger of a syphilitic patient in the secondary stage of the disease became infected and a typical chancre resulted. Gueyrat (74, 75, 76) made nineteen autoinoculations within eleven days after the appearance of the primary lesion. Taylor was able to observe positive takes fourteen days after the initial lesion appeared.

Levaditi, Laroche, and Yamanouchi, in studying the relation of the appearance of skin immunity during the primary stage of syphilis to the Wassermann reaction, found that inoculation was successful as long as the serum remained negative. As the disease becomes generalized the Wassermann reaction becomes positive and superinfection is no longer followed by a typical lesion.

While the initial lesion produces a non-susceptibility of the skin, this resistance to autoinoculation does not prevent the appearance of syphilitic manifestations during the later stages of the disease.

The spreading and increase of the virus giving rise to constitutional syphilis preclude the possibility of the presence of a general immunity. There are as yet no indications in support of the possibility that the mechanism of the skin manifestations during skin infection from without, differs from that which produces the skin lesions after the disease has become constitutional.

The non-susceptibility of the skin to superinfection appears to be well-marked during the secondary stage of the disease. The experiments of Finger-Landsteiner, which might give rise to doubts, are not sufficiently conclusive. The occurrence of the pallida in lesions resulting from the inoculation of non-syphilitic material for the purpose of controlling inoculation tests made on syphilitics in the secondary stage of the disease, as well as the fact that skin trauma may lead to the development of lesions containing the pallida at the point of injury, makes it impossible

to arrive at a definite point of view. We face the same condition when we consider the possibility of reinoculation during the tertiary stage of syphilis. It is evident from the descriptions that the lesions resulting from such attempts at reinoculation do not present the characteristics of a primary lesion. The reaction following the injection of killed cultures of the pallida into the skin of syphilitics seems to be similar to the reaction of the tissue subsequent to attempts at reinoculation.

Neisser (77), who has had the opportunity of studying this question on a large number of experimentally infected monkeys, found that animals which could not be reinoculated still harbored the organisms, and that animals which recovered, either spontaneously or due to therapeutic treatment, were susceptible to reinoculation.

In view of these experiments it is questionable whether the present conception of immunity applies to this disease. We may, in this condition, be dealing, on the one hand, with a lack of power on the part of the body to react to a new infection, or on the other, with an altered power of the tissues to react. The assumption that the virulence of the *Treponema pallidum* is lessened during its stay in the body, and that therefore the different lesions characteristic of the succeeding stages of the disease are the result, cannot be maintained in the light of experimental data. These data show that successful inoculations of the lower animals with tissues from lesions of any of the stages of the disease invariably produce lesions containing the organisms which in no way differ from those observed in the chancre.

We are thus led to the conclusion, as pointed out by Neisser, that not the invading organisms but the tissues of the body of the host are altered, reacting to the superinfection in the secondary stage, not with a primary lesion, but with a papule, and in the tertiary stage with a gumma.

That we are dealing with a changed condition of the tissues is furthermore brought out by the reaction following the intradermic injection of killed cultures of the pallida and their metabolic products.

Careful work over an extended period of time is required before this problem can even approach a conclusion. The work already done both in the laboratory and in practice has given us a new conception of the disease which is useful and stimulating.

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PRURITUS ANI.

PRELIMINARY NOTES.

CLINICAL FINDINGS.

BY LOUIS J. HIRSCHMAN, M.D., F.A.C.S.

BACTERIOLOGICAL FINDINGS.

BY HERBERT C. WARD, M.S.

(From the Research Laboratories of Parke, Davis & Company, Detroit, Michigan.)

All that I wish to present to the society to-day is the report of Mr. H. C. Ward, bacteriologist, who has been working on the bacteriology of pruritus and with a view to checking up the well known and scientific work of our colleague, Dr. D. H. Murray.

We started the latter part of December, 1915, and made cultures from twenty-five patients suffering from pruritus ani. The results of these cultures are herewith presented.

It will be seen from our work that the streptococcus fecalis has been found in a larger proportion of cases of pruritus in our investigations than even by Dr. Murray, himself. Unfortunately, however, our clinical results have not been as brilliant from the use of vaccines, either autogenous or polyvalent, as have been Dr. Murray's, but undoubtedly the fault was our own, if his contentions were correct.

In only four of our twenty-five cases did we have either a partial or complete cure from the vaccine treatment alone. In the other cases our results were achieved by the correction of the local pathological conditions which we found on examination in the majority of cases, or in the corrections of dietary errors, or the regulation of the patient's personal hygiene.

In another year I hope to make a further report on the therapy of vaccines, but at present must rest content with the report of Mr. Ward's work on the bacteriology of the streptococcus fecalis, which was carried on in conjunction with my work on the clinical side.

The bacteriological work was centered upon the isolation, cultivation and autogenous vaccine preparation of the etiological factor in this trouble. Dr. Murray has reported successful isolation in about 100 cases. No attempt, therefore, has been made

to make any further etiological search since the streptococcus fecalis appeared in our preparations.

Twenty-four cases have been diagnosed as typical pruritus ani cases by Dr. L. J. Hirschman, at whose office material was collected for the bacteriological isolation and vaccine preparations.

Areas showing pronounced infection were sterilized and then rubbed with cotton swabs. These were placed at once in tubes of plain broth and, after transference, incubated at 37° C. for 24 hours. Microscopical and stained preparations were then made and the record written. Subcultures were made on slant ascitic agar tubes, and after reincubation, typical streptococcic colonies were easily subcultured. Autogenous vaccines were made by using normal salt suspensions of the respective strains, killing cultures by heat, making standard suspension strengths, filling, and checking sterility.

Dilution strengths of these vaccines were at first 500 million per cubic centimeter, then 1000, 2000 and finally 4000 million organisms.

The following table includes a list of the bacteriological findings together with a record of the autogenous vaccines which were made:

VACCINES—TABLE I.

Record to June 1, 1916.

Case No.	Date Rec.	Bacteriological Findings.	Vac. No.	Strength
1	1-3-16	Strep. F. pred., Staph. Aureus, B. Coli....	031597	500 mil.
			091868	500 mil.
			091921	1000 mil.
			091958	2000 mil.
2	1-3-16	Strep., Staph., B. Coli, B. Proteus.....	None	
3	1-3-16	Strep. 5% B. Coli 95% long and short chains.	031596	500 mil.
			091807	500 mil.
4	1-4-16	Strep. long chains pred. B. Coli.....	031595	500 mil.
			091866	500 mil.
5	1-4-16	No. Strep., diplococcus P. G.....	None	
6	1-4-16	Strep. F. pred., Staph.....	031594	500 mil.
			091865	500 mil.
7	1-18-16	Strep. F., Staph. Aureus B. Spore bearer-	091879	500 mil.
		pred.	091909	1000 mil.
8	1-7-16	Strep. pred.	091880	500 mil.
9	1-8-16	Strep., B. Coli., B. Subtilis.....	091881	500 mil.
			091979	2000 mil.
10	1-10-16	Strep. 95%, Staph.....	091877	500 mil.
11	1-10-16	Strep. pure in scrapings, B. Coli.....	091878	500 mil.
12	1-17-16	Strep. and B. Coli.....	091898	1000 mil.
13	2-1-16	Strep. 95% and Staph.....	091899	1000 mil.
			091980	2000 mil.
14	2-4-16	Strep. F., long chains also, B. Coli	091900	1000 mil.
			091947	2000 mil.
15	2-10-16	Strep. F., B. Coli-pred.....	091908	1000 mil.
			091948	2000 mil.
16	2-10-16	Strep. F. 70%, B. Coli and Staph.....	091907	1000 mil.
			091957	2000 mil.
17	2-26-16	Strep. F., B. Coli-pred.....	091910	1000 mil.
			091943	2000 mil.

VACCINES—TABLE I—Continued.

Case No.	Date Rec.	Bacteriological Findings.	Vac. No.	Strength
18	2-28-16	Strep. 75%, B. Coli	091911	1000 mil.
			091946	2000 mil.
			091981	2000 mil.
19	2-28-16	Strep. F. 80%, Staph	091918	1000 mil.
20	4-24-16	Strep. and Staph	091991	4000 mil.
21	4-26-16	Strep., B. Coli, and Staph	091997	4000 mil.
22	4-26-16	Strep., B. Coli and Staph		
23	4-27-16	Strep. 95%, Staph. in one tube	091998	4000 mil.
24	4-27-16	Strep., B. Coli, and Staph	091999	4000 mil.
25	5-19-16	Strep. in one tube only B. Coli, pred.	092018	4000 mil.
26	5-31-16	Strep. pred. B. Tetanus? Also Coli		

The bacteriological work upon which Murray has based his conclusions was carried out by Dr. F. M. Meader and Dr. Bristol, of the Medical College, Syracuse University. The streptococcus identified by them is referred to as the *mannite fermenting streptococci.* They also report a lowered opsonic index to this class of streptococci as compared to staph. aureus of homologous cases.

By using a series of differential tests the majority (15 out of 17 tested) of the streptococci of the preceding table have been clearly identified as *mannite fermenting streptococci.* These strains answer all the requirements for this species according to the Gordon fermentation set and the characteristics as given by Andrews and Horder in their very complete report.

The main cultural characters of streptococcus fecalis, together with the fermentation differentation from other streptococci, are tabulated as follows:

FERMENTATIONS—TABLE II.

	Strep. Equinus Long Chains.	Strep. Mitis Short Chains.	Strep. Pyogenes Long Chains.	Strep. Salivarius Short Chains.	Strep. Angiosus Long Chains.	Strep. Fecalis Short Chains.
Temp.	20 —	20 +	20 +	20 +	20 —	20 +
Neutral red	—	—		+	+	+
Milk	—	Acid.	Acid.	+	+	+
Saccharose	+	+	+	+	+	+
Lactose	—	+	+	+	+	+
Raffinose	—	—	—	+	+	—
Inulin	—	—	—		—	
Salicin	—	+	+	—	—	+
Coniferin	+	—	—	—		+
Mannite	—	—	—	—	—	+
Pathogenicity	+	—	+	—	+	—
Broth	+	+	+	+	+	+
			wooly		wooly	turbid
Hemolysis	—		++	—	++	—

Streptococcus fecalis is further described by Horder and Andrews as being the most common from the point of distribution, the most resistant to unfavorable conditions, and the least pathogenic of the streptococcus species. It occurs as an oval celled streptococcus staining Gram positive and occurring usually in short chains. Growing at 20° and 37°, producing uniform

turbidity with hydrogen sulphide in broth cultures. By Schötmuller's blood test, it appears non-hemolytic and inactive. Action on raffinose and inulin is negative, while mannite is always fermented. A few varieties appear to liquefy gelatin, and most of the strains are non-pathogenic for rodents.

Agglutinations.—Table III.

Antistreptococcic serum 030245 Antigen, fresh susp. from an 18-hour growth.

Technic as usual incu. at 37°—24 hours.

Read after 24 hours incu. 6-4-16.

Cultures	1-50	1-100	1-200	1-500	1000	2000	Control
6	+	+	+	—	—	—	—
1	+	+	+	—	—	—	—
10	+	+	+	+	+	+	—
11	+	+	+	—	—	—	—
15	+	+	+	+	+	—	—
24	+	+	+	+	+	—	—
18	+	+	+	+	+	—	—
24	+	+	+	—	—	—	—
9	+	+	+	+	+	—	—
13	+	+	+	+	+	—	—
0203	+	+	+	+	+	—	—
Str. Equi	+	+	+	—	—	+	—

Conclusion. Standard Antistreptococcus Serum agglutinates type strains of str. fecalis up to 500.

Proteolytic Test.—Table IV.

When tested on gelatin, the following results were obtained.

Cultures inoculated into tubes of stock gelatin, and incubated for three days at 37° C.—when records were made.

Pruritus—Cultures.	Gelatin—Liquefied.	Collected Culture.	Gelatin—Liquefied.
1	—	0244	—
3			
4	—	0203	—
6	—		
7	—	0201	—
8	—		
9	+	0204	—
10	—		
11	—	0245	—
12	—		
13	—	0615	—
14	—		
15	+	0202	—
16	—		
17		P. A. R. I.	—
18			
19	—		
20			
21			
22			
23	—		
24	—		
25	—		
26	—		

Conclusion. 3 out of the 24—liquefy gelatin.

Hemolysis Test.—Table V.

In order to determine their action on blood the following tests were made.

Rabbits' blood was spread over glucose agar plates and then diluted cultures were added and the plates incubated for 24 and 48 hours.

The results are reported as follows:

Strain.	Hemolysis.	Viridans.
P. A. I.	—	—
3	-	+ Distinct
4		
6	+ Slight action	
7	—	—
8		+ Distinct
9	+ Distinct	
10	—	—
11	-	—
12	- Fair	-
13	+ Distinct	-
14	—	—
15	-	
16		-
17		
18	- -	
19	- -	
20	—	+ Distinct
21	—	—
22	-	-
23	—	
24	-	
25		
0615	+ Fair	-
0244	-	
0245	+ Fair	
R. P. A. 1		
Hem. Str. 3 (control)	+ Distinct	—

Conclusion. 16 cases of the 23 showed negative reactions.

One of the most recent reports is that of I. J. Kigler,* who summarizes his findings upon the differentiation of streptococci as follows:

"Sixty strains of streptococci from various pathological conditions were studied with respect to their agglutinative and fermentative properties.

The agglutination reaction was not found to separate the streptococci into large groups. However, by its correlation with the fermentation reactions, the probable relationship of these types is indicated.

The agglutination tests tend to show that a division of the streptococci on the basis of hemolysis is not warranted, whereas a separation according to the fermentation reactions appears to coincide more closely with their natural relationship.

*Ref. *Jour. of Inf. Diseases*, Vol. 16, 1915. Agglutination and Fermentation Among Streptococci.

The groups suggested are:

Str. pyogenes.—Salicin fermenters, which do not ferment raffinose or mannite, are generally hemolytic, and strongly pathogenic.

Str. salivarius.—Raffinose fermenters usually ferment salicin but do not ferment mannite, generally produce a green colony on blood agar, and usually cause subacute and chronic infections.

Str. fecalis.—Mannite fermenters generally ferment salicin, rarely ferment raffinose, and are variable in their reaction to blood and in their pathogenicity.

Resume of Findings.

1. Of our 24 cases studied, Streptococci have been isolated in 100%.

2. Seventeen cultures out of the 24 isolated have been studied, not only by morphological and biochemical tests, but also serologically.

3. 88% of these groups studied belong to the streptococcal species spoken of as *Fecalis,* as identified by the fermentation test.

COMPARATIVE RESUME.

Murray Cases 98.	Hirschman Cases 25.
ORGANISMS PRESENT.	ORGANISMS PRESENT.
Streptococcus, 86%	Streptococcus,100%*
B. Coli types, 57%	B. Coli types, 69%
Staphylococcus, 27%	Staphylococcus, 54%
Uncertain types, 15%	Uncertain types, 20%

Additional work is under way and the more complete report will discuss the value of vaccine therapy.

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*Streptococcus Fecalis was identified in 88% on a cultural differentiation. On general morphological identification 96% was so classified.

REPRINTS OF PUBLICATIONS FROM THE RESEARCH LABORATORY, PARKE, DAVIS & CO., DETROIT, MICH.

The present system of collecting reprints of articles published from the Research Laboratory was begun in 1912. Reprints of the following articles published subsequent to that time are available and will be sent upon request. The publications marked (*) are no longer available.

1. On the Administration of Diphtheria Toxin in a Collodion Sac. By E. C. L. Miller. (*Journal of Infectious Diseases,* Vol. 8, January, 1911, pp. 50-65.)

2. A Further Contribution to Our Knowledge of Insecticides—Fumigants. By Chas. T. McClintock, H. C. Hamilton and F. B. Lowe. (*Journal of the American Public Health Association,* Vol. 1, April, 1911, pp. 227-238.)

3. Duboisia Hopwoodii—A Histological Study. By Oliver A. Farwell. (Reprinted from *Merck's Report,* Vol. 20, May 1, 1911.)

*4. Etiology of Canine Distemper. By Newell S. Ferry. (*Journal of Infectious Diseases,* Vol. 8, June, 1911, pp. 399-420.)

*5. The Resistance of Smallpox Vaccine to the Coal-tar Disinfectants. By Chas. T. McClintock and Newell S. Ferry. (*Journal of the American Public Health Association,* Vol. 1, June, 1911, pp. 418-419.)

6. Production of Immunity with Over-Neutralized Diphtheria Toxin. By Chas. T. McClintock and Newell S. Ferry. (*Abdruck Aus Dem Centralblatt für Bakteriologie, Parasitenkunde und Infectionskrankheiten,* Abt. 1, Originale, Bd. 59, July 15, 1911, pp. 456-464.)

7. Soaps from Different Glycerides—Their Germicidal and Insecticidal Values Alone and Associated with Active Agents. By H. C. Hamilton. (*Journal of Industrial and Engineering Chemistry,* Vol. 3, August, 1911, pp. 582-584.)

*8. The Sleepy Grass of New Mexico: A Histological Study. By Oliver A. Farwell. (*Merck's Report,* Vol. 20, October, 1911, pp. 271-273.)

*9. Some Observations on the Physiological Action of Sleepy Grass. By A. W. Lescohier. (*Merck's Report,* Vol. 20, October, 1911, pp. 271-275.)

*10. An Investigation of the Depressor Action of Pituitary Extracts. By Carey P. McCord. (*Archives of Internal Medicine,* Vol. 8, November, 1911, pp. 609-620.)

11. The Physiology of the Pituitary Gland and the Actions of Its Extracts. By Carl J. Wiggers. (*American Journal of Medical Sciences,* Vol. 141, April, 1911, pp. 502-515.)

12. A Physiological Investigation of the Treatment of Hemoptysis. By Carl J. Wiggers. (*Archives of Internal Medicine,* Vol. 8, 1911, pp. 17-38.)

13. Notes on Catgut Sterilization: A Preliminary Report. By Willard H. Hutchings. (*Annals of Surgery,* Vol. 54, July, 1911, pp. 693-695.)

14. The Relations of Pyogenic Microorganisms to the Etiology and Treatment of Skin Diseases. By Henry Rockwell Varney. (*Ohio State Medical Journal,* December, 1911.)

15. A Micrococcus with Unusual Characteristics as a Factor in a Resistant Dermatosis Resembling Acne Vulgaris. By Henry Rockwell Varney and L. T. Clark. (*Journal of Cutaneous Diseases,* Vol. 30, February, 1912, pp. 72-78.)

16. Serum Treatment of Hemorrhage and Blood Dyscrasias. By A. W. Lescohier. (*New York Medical Journal,* Vol. 95, February 3, 1912, pp. 223-229.)

*17. Further Studies on the Bacillus Bronchicanis, the Cause of Canine Distemper. By Newell S. Ferry. (*American Veterinary Review,* Vol. 41, April, 1912, pp. 77-79.)

18. The Pharmacopœial Requirements for Cannabis Sativa. By H. C. Hamilton. (*Journal of the American Pharmaceutical Association,* Vol. 1, March, 1912, pp. 200-203.)

19. The Heart Tonic Unit. By H. C. Hamilton. (*American Journal of Pharmacy,* Vol. 84, March, 1912, pp. 97-103.)

20. Studies on the Etiology of Equine Influenza. By Newell S. Ferry. (*Veterinary Journal* (London), Vol. 19, April, 1912, pp. 185-197.)

21. A Method for the Bacteriological Standardization of Disinfectants. By Tatsuzo Ohno and H. C. Hamilton. (*American Journal of Public Health,* Vol. 2, May, 1912, pp. 331-338.)

22. Physiological Testing. By E. M. Houghton. (*American Druggist,* July and September, 1911, and January and April, 1912.)

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31. Studies on Hog Cholera. By Walter E. King and Robert H. Wilson. (*Journal of Infectious Diseases,* Vol. 11, Nov., 1912, pp. 441-458.)

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34. The Iodine Content of the Small, Medium and Large Thyroid Glands of Sheep, Beef and Hogs. By T. B. Aldrich. (Original Communications, Eighth International Congress of Applied Chemistry, Vol. 19, 1912, pp. 9-14.)

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THE OPHTHALMIC TEST FOR GLANDERS: WITH A SIMPLIFIED METHOD OF PROCEDURE.

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According to the Report of the Special Committee for the Detection of Glanders[1] which was presented to the fiftieth anniversary meeting of the American Veterinary Medical Association at New York, September, 1913, the following methods of testing for glanders were tabulated:

"Mallein Test.
- A—Subcutaneous.
- B—Ophthalmic.
- C—Cutaneous.
  - 1—Cutaneous.
  - 2—Dermal.
  - 3—Endermal.

Laboratory Diagnostic Methods.
- A—Examination of Pus or Nasal Discharge.
  - 1—Microscopic.
  - 2—Cultural.
  - 3—Animal Inoculation.
- B—Examination of Blood.
  - 1—Opsonic Test.
  - 2—Conglutination Test.
  - 3—Precipitation Test.
  - 4—Agglutination Test.
  - 5—Complement Fixation Test."

This list includes all of the known tests, that are considered of any value, both for field and laboratory work, irrespective of their relative merits.

While it is recognized that some of the above mentioned laboratory diagnostic methods are absolutely reliable and should always be used in obscure cases or for corroborative purposes, yet it is obvious that they can be carried out only in the laboratory by trained workers.

Therefore, for general diagnostic work and for field tests, one or more of the mallein tests must be resorted to.

Mallein, the substance found in cultures of the *Bacillus mallei* which is responsible for the supposed allergic reaction in animals properly sensitized, was first discovered by Hellman and Kalning in 1891. The exact composition of the active principle of mallein, whether it be a toxin or a proteid extract, still remains unknown, although its specificity has been established beyond a doubt.

Raw or concentrated mallein, as it is now prepared, is a dark brown syrupy fluid possessing a distinct characteristic odor and usually giving a neutral or acid reaction. Originally it was made by extracting potato cultures with water or water and glycerin, but at the present time the method of Roux is followed, namely: by growing one or more tested strains of *B. mallei* in glycerin peptone bouillon at 37.5° for about six weeks. At this stage the culture is usually sterilized by the addition of a sufficient amount of trikresol or some other preservative and filtered; after which procedure the filtrate is concentrated to one-tenth of its original volume with as little application of heat as possible.

During the past few years extensive experiments have been carried on in several countries to determine, if possible, which of the mallein tests would give the most satisfactory results for the general diagnosis of glanders.

According to Mohler and Eichhorn[2] "In judging a method which would be the most satisfactory for the diagnosis of glanders, various things have to be taken into consideration, but especially the reliability of the test. It should be convenient, the results should be manifested as early as possible, the reaction should be distinct and well marked, and, probably the most important of all, it should be possible for the practicing veterinarian to apply the test. The last condition must be seriously considered since the standing of the veterinarian in the community and the confidence of the public in his work would be more manifest if in suspected cases he could personally decide on the diagnosis instead of having to depend entirely on the results of serum tests made at some distant laboratory."

It seems to be the consensus of opinion among most of the authorities, both in this country and abroad, that the subcutaneous mallein test is not as reliable as was first thought and that the ophthalmic test is by far to be preferred.

According to Mohler and Eichhorn[2] "There is no question but that the subcutaneous mallein test is one of the valuable diagnostic agents for glanders, but no one can any longer deny that failures from this test are more numerous than are desirable. As a matter of fact, the uncertainty of the results from this test caused numerous investigators to seek some other methods which might replace the subcutaneous mallein test. Besides the failures resulting in this test, the technic of execution of the test, together with the time required for the conclusion of the test, makes it unpopular for many veterinarians and sanitary officers."

In favor of the ophthalmic test the following authorities are quoted: Mohler and Eichhorn[2] "The popularity of the test is rapidly gaining wherever it has been applied, and among its supporters we find at the present time the greatest authorities on the subject of glanders and on clinical diagnosis."

"Its practicability is apparent, and its use in the control of glanders appears to be now an absolute fact." The method was thoroughly tried out by the Bureau of Animal Industry and from reports in more than 18,000 cases the results from all sources were uniformly satisfactory.

The test has been officially recognized in several of the foreign countries as well as in Canada and in the United States. In a report of Mohler and Eichhorn[2] they say, "In the United States the Bureau of Animal Industry, in consideration of the favorable results obtained, has recognized the method of diagnosis for interstate shipments of equines."

Schnurer, probably the greatest authority on glanders, gives the following report:[10]

"During the period 1910-1913, 93,352 ophthalmic tests were carried out in Austria (excluding Galicia and Bukowina); out of these 341 glandered horses gave positive results in 88.8 per cent of cases, doubtful results in 7.6 per cent and negative results in 3.5 per cent. Out of 75,879 healthy horses 99.6 per cent gave negative reactions and 0.34 per cent positive reactions. The negative results in the glandered horses (3.5 per cent) are attributable in part to the fact that the horses were not only tested once or were tested only a few days before death, and probably in part to errors in judgment and mistakes in the post-mortem diagnosis."

He also says, (1) "Glanders can be stamped out by the slaughter of clinically affected animals and of animals recognized as diseased by means of a test. Immunization is at any rate superfluous.

"(2)—The most satisfactory test is one that does not involve the intervention of a central authority, yields reliable results within a short time (12 to 24 hours) in the hands of persons who are not required to be specialists, is easy to apply and to base a decision upon, is suitable for application on a large scale on the frontiers, can subsequently be verified, and is comparatively cheap.

"(3)—The serological tests (agglutination, complement fixation, precipitation, conglutination, Abderhalden's test and anaphylactic reaction) do not fulfill these conditions either singly or in combination with each other, because they cannot be carried out without the intervention of a central authority.

"(4)—The ophthalmic mallein test (conjunctival reaction) carried out with a reliable concentrated mallein painted upon the eye with a brush, swab, glass rod, or some other instrument, and not dropped into it with a pipette or drop bottle, satisfies all the conditions mentioned."

In the Report of the Special Committee for the Detection of Glanders, mentioned previously, the following may be found: "In deciding upon a method which would be most satisfactory for the diagnosis of glanders, the simplicity and trustworthiness of the method must be above reproach.

"The results should manifest themselves as soon as possible, the reaction should be well marked and distinct and easily applicable by the average practicing veterinarian.

"A test with these requirements places a test into the hands of the practicing veterinarian along with which the standing of the veterinarian in the community and the confidence of the public to the veterinarian is brought into closer relationship, in that it enables the veterinarian to personally decide on the results of the test.

"The ophthalmic test not only meets all these requirements, but is without doubt the most convenient diagnostic method at our command.

"Its reliability compares favorably with any of the other available tests.

"The reaction is usually distinct, and doubtful or atypical reactions are rather infrequent.

"The ophthalmic test does not interfere with subsequent serum or other mallein tests if such are deemed necessary.

"The ophthalmic test should be recognized by State and Federal authorities since its reliability can no longer be doubted.

"In all atypical and doubtful cases of the ophthalmic test the combined complement fixation and agglutination or subcutaneous mallein tests should be utilized for confirmation. Such a procedure should minimize the failures and assure the best results in the control of the disease in a single stable or in an entire community."

A comparative investigation concerning the various biological methods of glanders diagnosis was undertaken, in Russia, by a committee under the direction of Prof. Dedjulin on 245 healthy and 6 glanders-infected horses, with the following results:

Ophthalmic reaction	0 positive (0% failure in reaction)
Competent fixation method	0 positive (0% failure in reaction)
Agglutination	4 positive (2% failure in reaction)
Subcutaneous mallein reaction	4 positive (2% failure in reaction)
Precipitation reaction	11 positive (5% failure in reaction)

The ophthalmic and complement fixation reactions proved the most reliable methods in healthy horses.

In the infected horses all the methods gave positive reactions.

Dedjulin thus summarizes the results of his investigation, "That the malleinization (ophthalmic reaction) is to be regarded as the most efficient and for practice the most convenient aid for the diagnosis of glanders."

"It apparently yields no more failures in diagnosis than other methods, but it is decidedly simpler, and its execution can take place independently of the laboratory; this latter is of no little practical significance. Moreover, the judgment of results of this reaction seldom offers occasion for disagreement in opinion."

The writer, in preparing the material for the simplified ophthalmic test, followed the work of Foth[4], Wladimiroff[5], Fröhner[6], Reinhart[7], Meissner[8], and others, who used a desiccated precipitated mallein. This was made up by them into a watery solution and used in a similar way to the raw mallein, with like results. Comparing the dry purified mallein with the raw mallein, Mohler and Eichhorn[2] state that "The advantages

of the use of one as compared with the other of these forms of mallein for the eye are not marked, as equally good results were obtained from the application of both forms of this product."

The usual method of preparing the desiccated mallein is to precipitate the raw mallein with several volumes of absolute alcohol, wash the precipiate with ether and dry *in vacuo*.

Taking this as the point of departure from all preceding methods, the writer moulds the purified mallein with milk sugar, which is a soluble, non-irritating and harmless base, into small tablets, in such a proportion that each tablet shall contain the exact amount of mallein required for one test. Instead of dissolving the tablet in water prior to its application, as has previously been done with desiccated mallein, the tablet is placed directly into the conjunctival sac at the inner canthus of the eye and there allowed to remain. The tablet will soon (one to three minutes) dissolve without apparent discomfort or annoyance to the animal and without an irritating effect upon the conjunctiva. The mallein which is thus set free produces typical reactions similar to those recorded as the result of the instillation of the raw mallein, or the solution of the dried mallein.

Soon after this material was first prepared and tested, Meyer[9], from the Laboratory of the Pennsylvania State Livestock Sanitary Board, reported his results with desiccated mallein, which was the first report on the use of desiccated mallein in this country. Meyer prepared his "Mallein Siccum" by precipitating the raw concentrated mallein with 30 parts of absolute alcohol. The writer, who used a much smaller percentage of alcohol, has found that it is not necessary nor desirable for practical purposes to use as much as 30 parts of alcohol.

Meyer concluded after a thorough test of 210 horses with his desiccated mallein that "The conjunctival test for glanders is very reliable. It can, in a short time, without large expense, be applied by every practicing veterinarian and will permit the untrained to make a diagnosis of glanders with the greatest possible accuracy."

The advantages of the method proposed by the writer, for the general diagnosis of glanders, are evidenced by the fact that it fulfills the requirements of a most satisfactory test as suggested by Schnurer, Mohler and Eichhorn and others, while

the material itself, being composed of desiccated mallein moulded into a convenient form, is extremely stable, can be handled with impunity and placed directly into the conjunctival sac without a previous solution in water.

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A SERO-ENZYME STUDY OF BACTERIAL PROTEINS.*

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Abderhalden's stimulating demonstration of the existence of sero-enzymes has reacted most effectively by opening up new lines of investigation in chemistry and serology. No advancement, however, has been made over the masterly alignment of facts set forth by Ehrlich in his effort to explain the operation of the laws governing immunity. The apparent ease with which Abderhalden's methods of dialysis could be applied to the needs of immediate diagnosis in pregnancy, has unfortunately led many of us to accept our demonstrations of enzyme specificity in other conditions regardless of consequences.

The rationale of the dialysis method is quite elementary and dependent upon the fact that protein molecules are, as a rule, colloidal in character and therefore retained by animal, plant, or physically produced membranes. On the other hand, numerous chemical units derived from these molecules are capable of passing such barriers. When proteins belonging to a definite chemical group are introduced into the body, it can be shown that they stimulate the development of correlative enzymes, and these in turn bring about the decomposition of the proteins into their dialyzable fractions.**

The report of previous attempts to prove this method valuable in the diagnosis of infectious diseases has stimulated the present inquiry. Do the conditions permitting the successful application of this method in the diagnosis of pregnancy exist during the progress of a bacterial infection? Our assumption maintains that infectious processes are accompanied to a certain degree by a protein invasion. Bacterial proteins stimulate the production of specific enzymes and these react in turn by lysis of the proteins. Each type of protein produces a specific enzyme which is capable of homologous action only, so that a certain protein undergoing pronounced lysis indicates the character of the infection and determines the diagnosis.

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**This experimental work was carried on nearly two years ago, and since then scientific opinion has suggested the probability that foreign proteins parenterally introduced undergo no lysis themselves, but act rather as keys to unlock enzymic decomposition of the protein constituent of the blood serum itself. The amino acids of our dialysates come, therefore, from the homologous serum and not from the bacterial proteins of our artificial infections.

In the present study rabbits were injected with various bacterial extracts, and two preparations of diphtheroid and gonococcal cells were employed.

The *first* preparation consisted of washed cells. Established strains of bacteria were cultivated on a suitable medium to a maximum growth, removed, and washed repeatedly until free from soluble proteins. These cells were then dried or held in heavy suspensions.

The *second* contained all the filterable products of bacterial growth, together with those present in the original medium and those broken down from the same. There was practically nothing of the same nature which made up the bulk material of the washed cell residue of the first lot. All these preparations were preserved with 0.2 per cent. trikresol, stored at 10° C., and with but one exception have remained sterile up to date.

Healthy male rabbits were selected as best adapted for the work, and duplicate animals used for each special series of cells, and filtrate preparations. Daily records of thermal and local reactions were kept. Injections began after a normal temperature average was recorded. Inoculations were made subcutaneously, and with three exceptions all of the animals survived the injection series. The final tolerance to large doses has been accepted as evidence of a certain degree of immunity.

The animals assigned to the study of the action of the diphtheria toxin received seven graduated injections during a period of twenty days. The first dose began with 0.05 c.cm. and the last injection amounted to 4.0 c.cm. The local reaction was slight at first, necrosis developed with the third injection, but with the sixth the reaction was negative. However, both animals lost weight gradually and died in eight weeks. Thermal reactions were clearly evident. In the animals receiving the washed cells, the first injections began with 10 mgrm., while the last was recorded at 500 mgrm. Marked congestion developed around the areas of injection, and subcutaneous nodules would often persist for a week or longer. No marked thermal reactions appeared at first, but toward the last week the temperature went up and remained high, gradually falling with cessation of the experimental treatment.

In the gonococcus series, we found that six injections were

given during a period of four weeks. The doses were graduated from 1 c.cm. (the same dosage applying to both preparations) to 7 c.cm. The rabbits treated with the filtrates showed only a mild congestion. During the last third of the experiment this increased in intensity, and around the areas of injection extensive open unhealing ulcers developed and remained so for three weeks following.

The gonococcus-cell duplicates showed greater variations in temperature than did those of the other series. The negative phases following immediately after the initial dose were quite noticeable. In one case death ensued two days after the third injection. The local reactions showed congestions and swellings, developing into open ulcers which refused to heal. This result was duplicated in the rabbits that had received the gonococcus filtrate preparations.

When we consider the method of determining the enzyme titre of the rabbits' sera, it is easily apparent that the results together with their interpretations are absolutely dependent upon the working conditions.

The rabbits were bled at 10:00 a. m., twenty-four hours since their last feeding. Samples of serum were obtained from two to five days following injections. Blood was taken from the ear veins in amounts of 5 to 10 c.cm., centrifuged, separated, and transferred to low temperature conditions until used at 5:00 p. m.

The bacterial proteins were prepared as follows: Mass culture growths were repeatedly washed in sterile salt solution and centrifuged until the supernatant liquid gave no reaction with the ninhydrin test. The sediment was then mixed with fresh solution to a uniform degree of density, measured by its gross appearance, fluidity in a fine pipette, and comparable amounts of weighed dried cells. To prepare the dried proteins, the bacterial sediment was evaporated at 37° C. for forty-eight hours, ground to a fine flour and stored under vacuum. The stability of these bacterial preparations was gauged from time to time.

In setting up the dialyzing test, standard parchment thimbles were placed in glass-capped tubes. A few c.cm. of sterile distilled water were added and covered with toluol. Measured amounts of serum were placed inside these thimbles and then the bacterial protein of the washed cells. This was overlaid with a toluol film.

Records of each thimble content combination were made and the series incubated at 37° C. for sixteen hours. The dialysates were then analyzed in duplicate sets, employing the ninhydrin reaction.

Mention can be made of only an important point or so of the technique. The method of heating the thimbles just before using and after each test was as follows: After washing, the thimbles were heated gradually to a temperature of 75° C. during a period of thirty minutes and then cooled during thirty minutes. Attention was thus given to graduating all changes of temperature with the idea that relative irregularities of expansions and contractions would find better adjustment. With such care, the parchment thimbles have retained their relative permeability during the course of some twenty dialyzing series.

A second point considers the ninhydrin test. Instead of using 5 c.cm. of dialysate plus 0.1 c.cm. of ninhydrin solution, 2 c.cm. of dialysate and 0.05 c.cm. of the reagent were found to yield comparable results. For uniform heating we have employed a glycerine bath maintained at a temperature of 130° C. The time of complete exposure was five minutes. By this means the control of the initial heating and the uniform concentration of the boiling solution were more easily accomplished.

For recording the various results of the tests, a scale of colors was prepared.

Standard dilutions of seiden peptone were made and with each dilution a triplicate set of ninhydrin tests. The means of these resulting shades of blue were used to determine the corresponding shade of neutral litmus solutions, and a series of tubes containing samples of these litmus solutions was made up. In our color standard, the most intense reaction corresponded to that obtained with the ninhydrin test upon a 1-1000 solution of peptone. This was number 1, and the series ran from 1 to 10, ten being absolutely colorless. The color reactions have been recorded by comparison of the test tubes with the standard series, as 1, 4, 7, 9, etc.

In studying the results obtained in the series of rabbits which had received injection of diphtherial proteins, it was noticed that in the majority of the records positive color reactions were nearly always present. Anticipation of such reactions clearly showed the need for a control of every test.

I.—TABLE OF COMBINATIONS.

	Amt. of Serum.	K. L. Protein.	Strep. Protein.	Results. 1-intense- Pos. 10-colorless-Neg.
Serum of Rabbit No. 505 Treated with K. L. Protein.....	0.3	.2	0	5
	0.3	0	.2	0
	0.3	0	0	0
Serum of Normal Rabbit No. 300.	0.3	.2	0	0
	0.3	0	.2	0
	0.3	0	0	0
K. L. Protein.....	0	.2	0	0
Strep. Protein.....	0	0	.2	0

Such a record indicates an ideal Positive Reaction. Contrast this ideal condition with an actual report.

II.—TABLE OF COMBINATIONS.

	Amt. of Serum.	K. L. Protein.	Strep. Protein.	Results.
No. 505 K. L. Serum.....	0.3	0.1	0	2
	0.3	0	0.1	5
	0.3	0	0	7
No. 300 Normal.....	0.3	0.1	0	2
	0.3	0	0.1	4
	0.3	0	0	8
K. L. Protein.....	0	2	0	0
Strep. Protein.....	0	0	2	0

In analyzing the results obtained in this series, we must bear in mind, therefore, that various degrees of reaction were always present. When the strength of the tests made from thimble sets containing K. L. proteins is compared with those of the control thimbles containing homologous serum only, there is present a suggestive increase in the relative strengths of the reactions in the protein thimbles. The amino acids produced in the protein sets are increased remarkably over those of the *serum alone* controls.

When the *normal serum* controls are considered, there is no increased titre of amino acids in the sets containing protein and the serum of untreated rabbits as compared to treated sera. When the control test on the protein of streptococcal cells with all the sera is compared, no specific titre can be observed.

In the records of the series of tests made upon the sera of these rabbits receiving injections of gonococcus proteins, a parallel study yields very comparable results. Increased attention to the technique makes this last series the most valuable. The percentage of error was greatly reduced, the reactions graded more carefully, and the results were satisfactory. The findings are, however, negative. Using the same tabulation, the same alignment of facts occurs. The tabulation of results obtained by one series in the case of the gonococcus washed-cell-proteins and the corresponding diphtheria set is here included.

III.—TABLE OF COMBINATIONS. (Gonococcus Proteins.)

Sera taken within 48 hours following 4th injection series.

Gon. Series (Washed cells.)	Sera Amt.	Gonococcus Protein.	K. L. Protein.	Ninhydrin Tests.	Notes.
No. 1. Rab. 518.....	0.3	0.5	0	6	
No. 2. Rab. 518.....	0.3	0.5	0	5	Duplicate
No. 3. Rab. 518.....	0.3	0	0	6	
No. 4. Rab. 517.....	0.5	0.2	0	7	
No. 5. Rab. 517.....	0.5	0	0.1	5	Protein Control
No. 6. Rab. 517.....	0.5	0	0	7	
(Filtered Protein)					
No. 7. Rab. 513.....	0.3	0.2	0	4	
No. 8. Rab. 513.....	0.3	0	0.1	8	Protein Control
No. 9. Rab. 513.....	0.3	0	0	6	
No. 10. Rab. 513.....	0.3	0	0	6	
No. 11. Rab. 520.....	0.3	0.2	0	5	
No. 12. Rab. 520.....	0.3	0	0	0	
(K. L. Washed Cells)					
No. 13. Rab. 505.....	0.3	.2	0	6	Non-specific
No. 14. Rab. 505.....	0.3	0	0	8	Serum Control
No. 15. Horse Serum.	0.4	0.2	0	5	Non-specific
No. 16.	0.4	0	0	6	Serum Control
No. 17. Control 1....	0	0.5	0	0	Protein
No. 18. Control 2....			0.1	0	Stability Test

A study of this reveals negative findings.

According to the results in Table III, the sera of these treated animals is no more active upon their specific bacterial substrates (see No. 4) than upon the other specific proteins (see No. 5). These sera are no more active on gonococcal proteins than is the sera of animals treated with a different protein (see No. 13).

Throughout the entire table, however, there is strong evidence of amino-acid formation taking place wherever serum and protein are in contact (see Nos. 5, 7, 11, 13). Various proteins therefore appear to be active on rabbit serum. For our main study, Table III shows negative findings.

IV.—TABLE OF COMBINATIONS. (Diphtheroid Protein.)

Sera taken five days following 5th injection series.

K. L. Series.	Sera Amt.	K. L. Protein.	Strep. Protein.	Ninhydrin Tests.	Notes.
No. 1. Rab. 500.....	0.2	0.2	0	6	Duplicate
(Toxins)					
No. 2. Rab. 500.....	0.2	0.2	0	6	
No. 3. Rab. 500.....	0.2	0	0	8	
No. 4. Rab. 504.....	0.1	0.2	0	6	Protein Control
(W. Cells)					
No. 5. Rab. 504.....	0.1	0.2	0.1	6	
No. 6. Rab. 504.....	0.1	0	0	8	
No. 7. Rab. 505.....	0.1	0.2	0	8	
(Duplicate)					
No. 8. Rab. 505.....	0.1	0	0	8	
No. 9. Rab. 514.....	0.1	0.2	0	5	Marked Positive Reactions
(Untreated)					
No. 10. Rab. 514.....	0.1	0	0	0	
No. 11. Rab. 525.....	0.1	0.2	0	4	Marked Positive Reactions
(Untreated)					
No. 12. Rab. 525.....	0.1	0	0	0	
No. 13. Control	1.0	0.2	0	0	Protein Stability
No. 14. Control	2.0	0	0.1	0	Protein Stability

Marked positive reactions obtained from the sera of untreated animals, Nos. 9 to 12, indicate the negative character of this entire series.

As in table No. 3, wherever serum and protein were in contact, as in thimbles Nos. 1, 2, 4, 5, 9 and 11, there is evidence of amino acid formation; whereas in control thimbles containing either bacterial protein or sera alone, specific or non-specific, no such formation developed.

In reviewing the entire series of this experimental work, it is evident that the conditions should permit of satisfactory conclusions. Bacterial proteins, specific biologically, together with their derivatives, have been carefully employed. Injections have simulated, to a certain extent, avenues of a natural infection, physiologically speaking. Susceptibility to and a definite degree of immunity against these proteins was to be found in the thermal and tissue reaction records. Control agglutination tests were 1-500 in the single case of the animals receiving the gonorrheal washed cells. Repeated sero-enzyme titres have not given definite evidence of any specific response on the part of the tissue to the introduction of the foreign proteins.

Such negative findings are consistent with the assumption that the sero-enzyme response becomes apparent when foreign proteins of a relative high constitutional complexity enter into an extensive physiological engagement with the tissues. By employing subcutaneous methods of injection, the field of action is greatly limited, and this is, we believe, comparable to natural conditions of infection in the case of diphtheroid and gonococcal infection. Proteins introduced in small amounts subcutaneously do not create a measurable sero-enzyme titre. High grade proteins introduced intravenously would be degraded theoretically through such a production. Low grade proteins introduced intravenously would not require the same enzyme activity to the same degree. Logically, they would require but a small fraction of that energy or even suffer further disintegration under the stimulus of common enzymes more or less inactive upon their previous structural units. If a given amino acid may be derived from the proteid constituents of egg albumin and also from those making up diphtherial cells for instance, and were we to introduce this common protein derivative into the tissues of an animal,

would the sero-enzymes developed be specific for egg albumin or for diphtherial protein, or for this split product only? Why should the tissues react to develop high grade enzymes for a low grade protein invasion? The logic of physiological economy suggests otherwise. Therefore, in view of the above incompletely

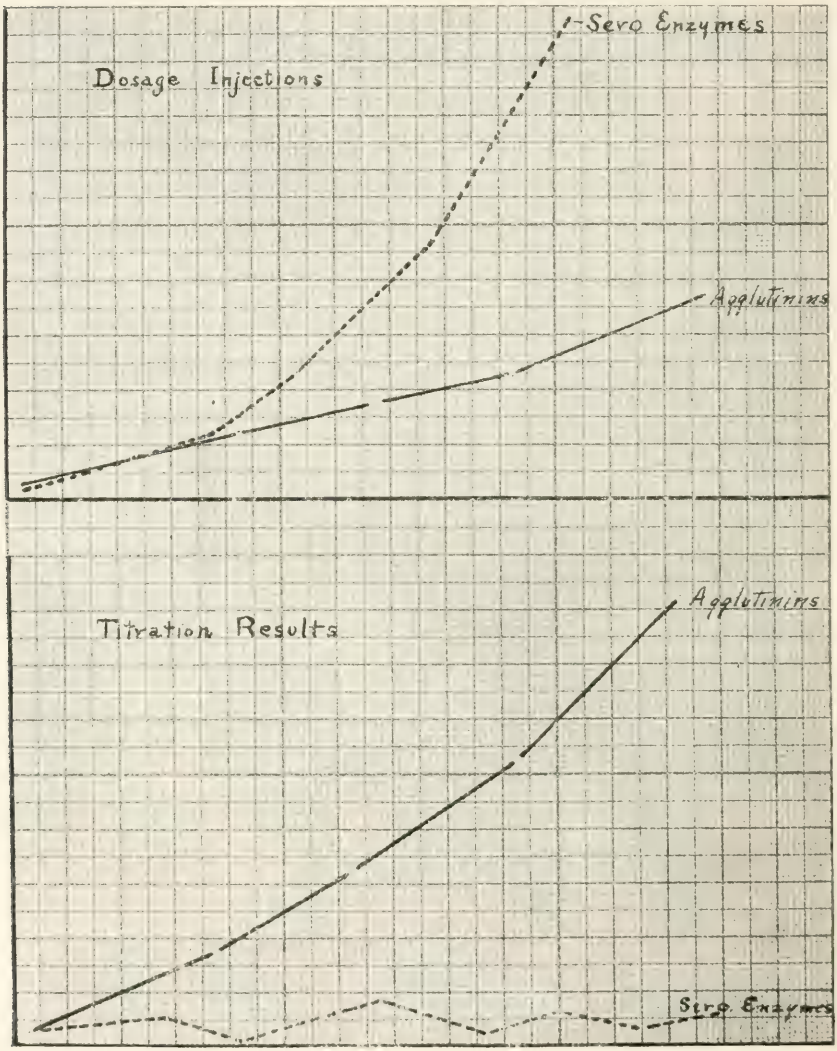

Chart I.—Illustration of Comparative Valuation of Serological Titrations.

expressed assumption, the negative results are consistent with the experimental work in our effort to call forth an enzymic titre from tissues which did not need such so-called protection.

We have observed that experimental animals have received increased amounts of proteins up to ten- and fifty-fold. After the first injection positive immunity has been established, but no measurable enzymic response has developed in proportion to the injection series.

Theoretically there should be such a response. This failure to demonstrate a proportionate development of sero-enzymes specific or a general development over the control titrations of untreated animals, stands in striking contrast to the bactericidal, agglutinative and opsonic titrations in experimental animals subjected to a parallel series of injections.

The conclusions of this study with reference to the purpose of taking it up, as outlined in the first inquiry, are as follows:

1. The sero-enzyme test does not appear from these experiments to be of diagnostic aid in such bacterial infections.

2. The conditions which are believed to exist in the sera of pregnant patients do not exist in any measurable degree in the case of diphtheria and gonococcus experimental infections in rabbits.

3. Neither proteins of a higher constitutional grade nor those less complex excite a measurable specific sero-enzyme response in rabbits.

REPRINTS OF PUBLICATIONS FROM THE RESEARCH LABORATORY, PARKE, DAVIS & CO., DETROIT, MICH.

The present system of collecting reprints of articles published from the Research Laboratory was begun in 1912. Reprints of the following articles published subsequent to that time are available and will be sent upon request. The publications marked (*) are no longer available.

1. On the Administration of Diphtheria Toxin in a Collodion Sac. By E. C. L. Miller. (*Journal of Infectious Diseases,* Vol. 8, January, 1911, pp. 50-65.)

2. A Further Contribution to Our Knowledge of Insecticides—Fumigants. By Chas. T. McClintock, H. C. Hamilton and F. B. Lowe. (*Journal of the American Public Health Association,* Vol. 1, April, 1911, pp. 227-238.)

3. Duboisia Hopwoodii—A Histological Study. By Oliver A. Farwell. (Reprinted from *Merck's Report,* Vol. 20, May 1, 1911.)

*4. Etiology of Canine Distemper. By Newell S. Ferry. (*Journal of Infectious Diseases,* Vol. 8, June, 1911, pp. 399-420.)

*5. The Resistance of Smallpox Vaccine to the Coal-tar Disinfectants. By Chas. T. McClintock and Newell S. Ferry. (*Journal of the American Public Health Association,* Vol. 1, June, 1911, pp. 418-419.)

*6. Production of Immunity with Over-Neutralized Diphtheria Toxin. By Chas. T. McClintock and Newell S. Ferry. (*Abdruck Aus Dem Centralblatt für Bakteriologie, Parasitenkunde und Infectionskrankheiten,* Abt. 1, Originale, Bd. 59, July 15, 1911, pp. 456-464.)

7. Soaps from Different Glycerides—Their Germicidal and Insecticidal Values Alone and Associated with Active Agents. By H. C. Hamilton. (*Journal of Industrial and Engineering Chemistry,* Vol. 3, August, 1911, pp. 582-584.)

*8. The Sleepy Grass of New Mexico: A Histological Study. By Oliver A. Farwell. (*Merck's Report,* Vol. 20, October, 1911, pp. 271-273.)

*9. Some Observations on the Physiological Action of Sleepy Grass. By A. W. Lescohier. (*Merck's Report,* Vol. 20, October, 1911, pp. 271-275.)

*10. An Investigation of the Depressor Action of Pituitary Extracts. By Carey P. McCord. (*Archives of Internal Medicine,* Vol. 8, November, 1911, pp. 609-620.)

11. The Physiology of the Pituitary Gland and the Actions of Its Extracts. By Carl J. Wiggers. (*American Journal of Medical Sciences,* Vol. 141, April, 1911, pp. 502-515.)

12. A Physiological Investigation of the Treatment of Hemoptysis. By Carl J. Wiggers. (*Archives of Internal Medicine,* Vol. 8, 1911, pp. 17-38.)

13. Notes on Catgut Sterilization: A Preliminary Report. By Willard H. Hutchings. (*Annals of Surgery,* Vol. 54, July, 1911, pp. 693-695.)

14. The Relations of Pyogenic Microorganisms to the Etiology and Treatment of Skin Diseases. By Henry Rockwell Varney. (*Ohio State Medical Journal,* December, 1911.)

15. A Micrococcus with Unusual Characteristics as a Factor in a Resistant Dermatosis Resembling Acne Vulgaris. By Henry Rockwell Varney and L. T. Clark. (*Journal of Cutaneous Diseases,* Vol. 30, February, 1912, pp. 72-78.)

PITUITARY STANDARDIZATION.

BY H. C. HAMILTON AND L. W. ROWE, DETROIT, MICH.

(From the Research Laboratory of Parke, Davis & Co., Detroit, Mich.)

The value of pituitary extract (posterior lobe) as an effective therapeutic agent for controlling blood pressure and for stimulating uterine contractions in the second stage of labor is universally recognized. Because of these important uses it is necessary that the extract be standardized as accurately as possible.

The crude materials from which valuable substances are obtained are unfortunately very variable. This, however, is of slight importance compared to the effect that the various chemical and pharmaceutical manipulations, incident to the preparation of these substances, have on their activtiy. The adoption of a high standard of activity and rigid adherence to the same is imperative for a product of such great physiologic importance as that of pituitary extract. In the case of this extract, as is also true of extracts of some other glands and crude drugs, the chemical constituent to which it owes its therapeutic value has not been isolated.

In such cases advantage is taken of the fact that active medicinal substances when administered to animals have characteristic physiologic effects which are more or less typical and which can be used as assay reactions. This procedure is similar to the use of chemical reactions on which to base chemical assay processes. Biologic standardization is now generally recognized as an indispensable adjunct to the commercial valuation and to the scientific investigation of certain substances of great importance in medicine.

When several physiologic effects are more or less characteristic of the substance in question, differences of opinion naturally prevail as to which is best adapted to an accurate quantitative assay process.

It is the intention of the authors to describe the two methods that have been proposed and used for the assay of the various substances and extracts derived from the pituitary gland, and to point out the advantages of each as these have developed under practical working conditions.

The two methods referred to above are those known as the blood pressure and the oxytocic tests.

In a former communication[1] some of the physiologic effects following the administration of extracts of the pituitary body were reviewed and discussed as to their adaptability to the purpose of standardization.

Of the various characteristic effects, that on the circulatory system was considered most constant and subject to the least variation, due to causes other than differences in the amount administered. This effect was, therefore, adopted as an assay reaction to determine the activity of solutions prepared directly or indirectly from the infundibulum of the pituitary gland.

THE BLOOD PRESSURE METHOD.

The method of assay in brief is as follows:: A dog weighing approximately 10 kilos is anesthetized, preferably with chloretone, and is prepared for test purposes by inserting a cannula into a femoral or other convenient vein for injecting the solution, and another cannula into one of the carotid arteries. By means of a flexible connection the artery is joined to a manometer attached to a kymograph, to obtain a record of the normal heart action and blood pressure for comparison with that following an injection of the active extract. The standard originally[1] used for comparison in making such assays was the activity obtainable from the dried, defatted, powdered, infundibular portion of the bovine glands. Gland material so prepared seems to retain its activity indefinitely and to permit of quick and complete extraction by simple infusion. Later, however, the possible objections to this have been obviated by the use of a stable and highly active water-soluble powder prepared by Aldrich.[2] This was carefully assayed and its activity compared with that of the former standard. The Standard Test Solution is prepared from this, using a sufficient quantity of the powder to make a solution containing in 1 c.c. the highest activity found in 1 mg. of the dried gland material referred to above.

The technic of the test is to inject the standard test dose—1 c.c of the Standard Test Solution—into the vein, note the rise in blood pressure, and after 15 minutes repeat the injection. It is sometimes necessary to repeat this injection two or three times

before one obtains equal changes in blood pressure from two consecutive injections of the test dose.

The standard and sample are then injected alternately with intervals not less than 15 minutes, varying the amount of the sample, if necessary, until the rise in blood pressure is the same from definite quantities of each. The activity of the sample can be deduced from the amounts used and is stated in terms of the Standard.

The following points must be carefully observed as a means of making this an accurate quantitative method for the valuation of pituitary extracts:

1st. The dog must be healthy and should maintain a good normal blood pressure.

2nd. It must be completely anesthetized until all reflexes are destroyed.

3rd. It must be sensitive to changes of 10 per cent in the amount of pituitary extract injected. For example, if doses of 0.9, 1 and 1.1 c.c. of the test solution induce the same amount of change in blood pressure with no measurable differences between them, that dog is not in a condition satisfactory for standardization work.

4th. It must be sufficiently sensitive to pituitary extract so that 1 c.c. or at most 2 c.c. of the Test Solution (q.v.) raises the pressure at least 1 cm.

5th. Two consecutive injections should have at least 15 minutes interval between them.

The method just described was adopted tentatively until a more logical or accurate one could be developed.

THE ISOLATED UTERUS TEST.

The increasing use of pituitary extract in obstetric work suggested a much more logical method of assay than the one described, namely, a test based on the action of pituitary extracts on the uterus muscle. Such a method was used and first described by Dale and Laidlaw[3] and afterwards by various investiators, notably Fühner[4] and Guggenheim.[5] The experimental work has been exhaustively summarized by Roth[6] who assayed eleven commercial samples and reported his results.

The great differences in activities of the samples examined

led him to the conclusion that "the statement 'physiologically standardized' means practically nothing." Roth, however, apparently overlooked the fact brought out by his own tests that in every case the two samples from the same manufacturer had the same activity, indicating that each laboratory although having a different standard had adhered to it.

The fault, if any existed, was not in the lack of a standard nor in a failure to apply standardization tests, but in the failure of the manufacturers to co-operate in choosing a single standard.

Roth, in a previous article[7] proposed the use of *B*-iminazolylethylamine, commonly called histamine, as a standard for measuring the activity of pituitary extracts. His proposal now reappears as the official standard for a U. S. P. solution of hypophysis extract and is compulsory to the extent that extracts standardized in any other way do not meet official requirements. While a very dilute solution is required for standardization purposes, the present almost prohibitive price of histamine, due to the fact that the supply in this country is very limited, tends to preclude its use as a standard.

Such a standard is also open to question from two view-points. Are different lots of histamine equal in their action on the uterus? Is a substance which has no practicable value in obstetrics,[8] when used alone, a true standard for determining the activity of a powerful oxytocic agent such as pituitary extract, and is it a proper standard when the action in question is as a pressor agent? If the value of pituitary extract were only as an oxytocic agent, or if only that portion applied to obstetrical purposes were to come under the U. S. P. ruling, then some of the objections to the method and the standard would be removed. Under present conditions, however, a more commendable standard would be a substance prepared from the gland and having the same qualitative action as fresh glandular extracts.

While the details of the technic and equipment for this test vary in different laboratories, the essentials are briefly as follows: One horn of the excised uterus of a young virgin guinea-pig is used, it being suspended between a fixed point, and the end of a movable lever. It is bathed in Locke's solution which is kept at a uniform temperature of 38° C. and constantly aerated with air or oxygen.

The solutions to be tested are mixed with the Locke's solution in which the muscle is suspended and only the small part in contact with the strip of uterus is responsible for the effect produced. The exquisite sensitiveness of this muscle to the action of stimuli such as an active pituitary extract, is shown by the fact that some specimens are sensitive and respond to the stimulus of the infinitesimal amount which comes in contact with them from a solution containing only 1 part in 100,000,000. This shows too the exceedingly powerful character of the active principle of the gland, for although the substance referred to above was highly purified, it did not possess the physical characteristics of, and probably was not a pure principle.

Without entering into a detailed description of the method by the authors, which is outlined above, some points will be especially considered because so much depends on the carefulness with which they are followed.

The Test Animals.—While differences of opinion exist as to the one best adapted, most investigators have agreed on the young virgin guinea-pig weighing 250 to 350 gms. and rejecting those in the œstral stage.

The Organ.—One horn of the uterus using a strip 1 to 2 cm. long. It should be normally slender and not congested. Congested, thickened uteri, while often very sensitive, are too irritable for standardization tests.

The Apparatus.—This includes the kymograph, a writing lever, a constant temperature bath accurately adjusted to hold a temperature of 38° C., an inner container for Locke's solution in which the uterus muscle is suspended, and facilities for preheating the Locke's solution to the same temperature as that of the bath, and for its aeration (see Fig. 1).

The heating of the constant temperature bath can be done accurately and conveniently by the method described by Dale and Laidlaw, a gas flame striking a copper rod which passes into and around the outer vessel. The apparatus in use at present and which has proved very satisfactory for maintaining a uniform temperature consists of a rectangular vessel of 8 liters capacity which is heated from below by an electric heating coil enclosed in a soapstone box. The amount of current is regulated by a rheostat and when once adjusted will maintain the temperature

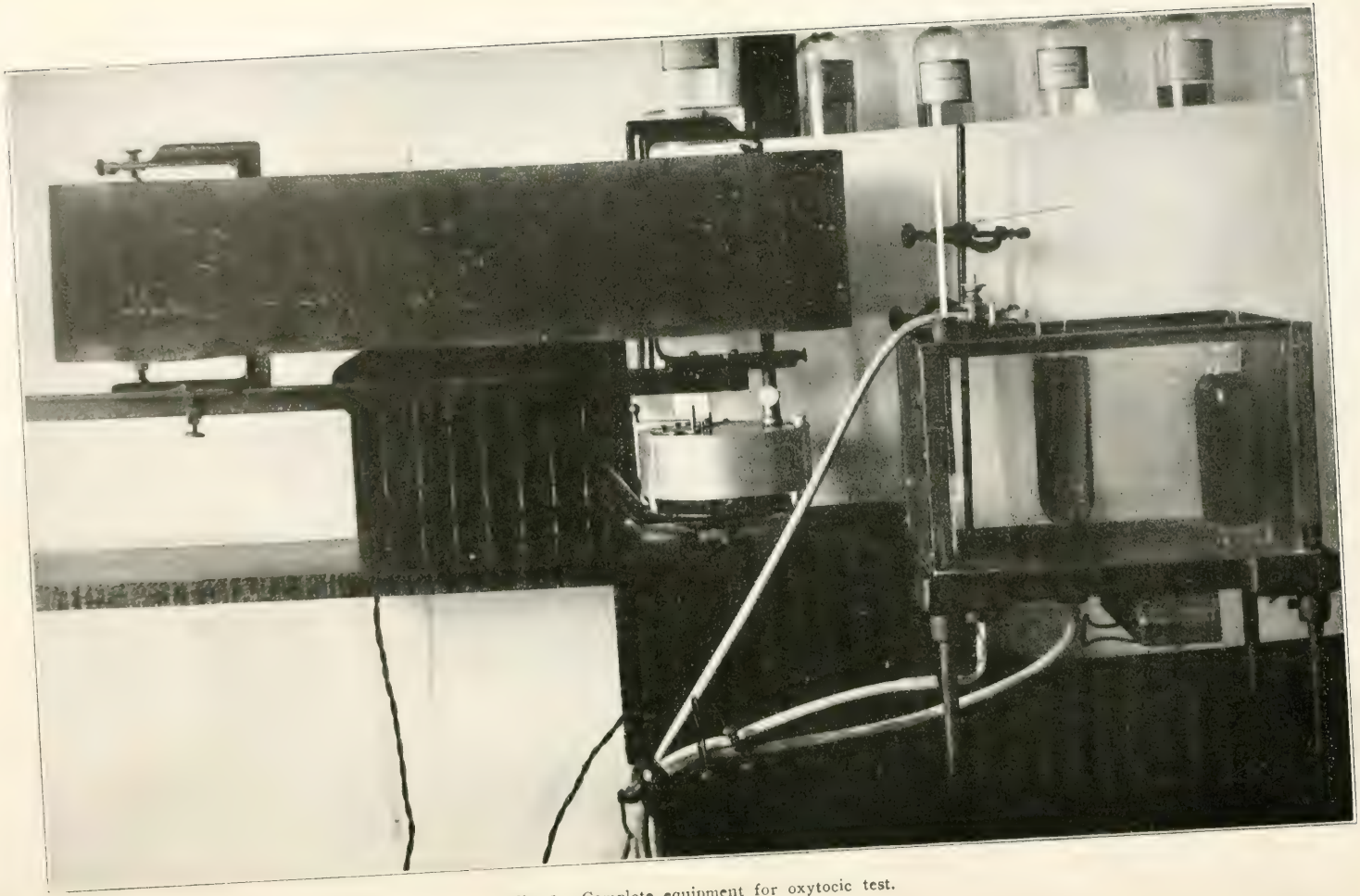

Fig. 1.—Complete equipment for oxytocic test.

of the water bath at 38° C. provided the room temperature remains fairly constant. The large outer vessel has a soapstone bottom covered inside with copper, while two sides are made of double walled glass with an air space between. The apparatus is well adapted to the purpose intended and is very simple in construction and operation.

The preheating of Locke's solution is most conveniently done by keeping a supply in another container in the outer bath, in which it attains and holds the same temperature as that in contact with the muscle. (Note bottle to right in large vessel.)

The aeration of the muscle is best secured by the method suggested by Dale and Laidlaw, conveying a current of air or oxygen through the tube (leading to top of inner container) which forms the lower support of the muscle, the upper end of the muscle being attached by thread or otherwise to the writing lever.

The writing lever should be light, such as a straw or an aluminum wire, should move almost without friction, and magnify the contractions 3 to 5 times.

Not of least importance is the Locke's solution, which should be made of the best quality "reagent" chemicals.

The Technic.—This includes weighting the muscle to counteract the excessive contractions of an irritable uterus; injecting into the Locke's solution quantities of sample and standard which will induce equal contraction of the uterus less than the maximum for the specimen; maintaining in the meantime a constant temperature, removing spent pituitary solution; washing the specimen, and repeating injections not too often to destroy its tonicity. Injections can best be made by means of a hypodermic syringe, which forces the dose into the solution and tends to mix and make the solution homogeneous almost immediately. This is aided by the continuous bubbling of air through the solution. When the maximum contraction for any dose has been reached and no further rise of the writing lever takes place, the spent solution is withdrawn through a valve at the bottom of the inner container passing through the bottom of the outer bath. The specimen is washed with fresh Locke's and an additional quantity is left in contact filling the container to some determined volume. Another injection can be made as soon as the muscle has relaxed to normal length.

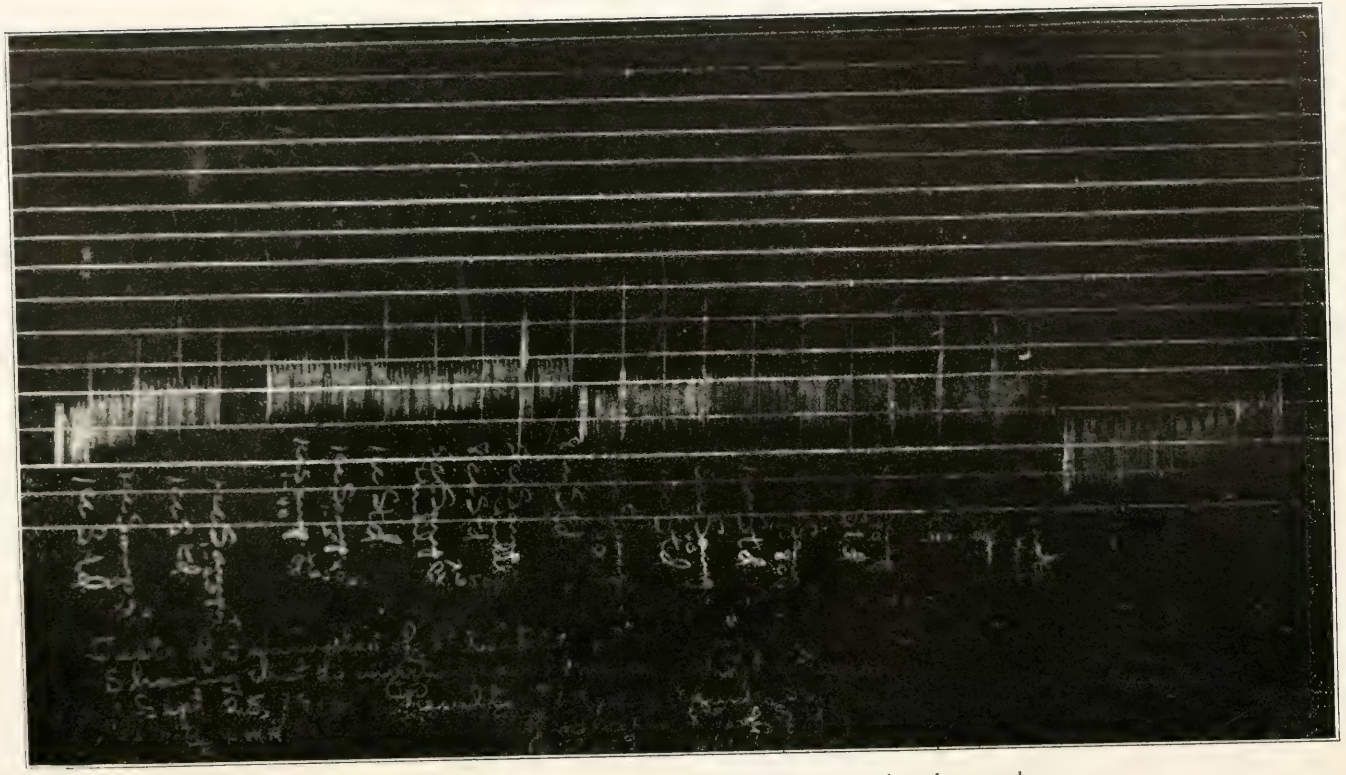

Fig. 2.—Twenty-four consecutive injections of pituitary extract into the same dog.

DISCUSSION OF METHODS.

The oxytocic test on the uterus muscle is without question more logical than the blood pressure effect for standardizing substances used almost exclusively as oxytocic agents, unless there is evidence that the same active constituent is responsible for both effects. If this is true in any particular instance, then accuracy and convenience only need be consulted. Opinions differ regarding this point as applied to the pituitary gland, but the most generally accepted one is that there are several active bodies with different physiologic actions. In other words an extract of the gland might raise the blood pressure without having any oxytocic activity. This is a point which requires more than passing consideration. Because of the fact that a good specimen of the isolated uterus of a guinea-pig is sensitive to a much smaller quantity of pituitary extract than can be detected by the blood pressure test, and that such a test organism is also very sensitive to other substances and to variations in the test conditions, it is very difficult to prove that there is both a pressor principle and an oxytocic principle in the glands. The authors have not yet found any data to support the contention that the two actions are not due to an identical substance. Any chemical treatment to isolate one from another either destroys both actions or affects them equally.

Another objection urged against the blood pressure method as of noteworthy importance,[6] is the statement that repeated injections of pituitary extracts, even when prepared from the separated infundibulum, induce progressively decreasing pressor effects until the depressor predominates and no rise in blood pressure follows the injection of the active extract. This, however, is not a valid objection when the test conditions outlined above are followed carefully.

In many cases between 20 and 30 doses of 0.05 c.c. Pituitrin or an equivalent quantity of similar preparations have been injected intravenously into dogs without the depressant effect becoming noticeably increased. (See tracing, Fig. 2.) If large doses were used and the injections followed each other closely the objection noted would be a valid one. All physiological testing, however, must be done under rigidly prescribed conditions, otherwise there is little accuracy.

In this method of testing, the test animal is the dog admitted by all to be the least subject to the so-called tolerance; the doses are small; they follow each other at intervals not less than 15 minutes; sample and standard are injected alternately and must give an equal rise in pressure; the rise in pressure from the standard test dose must be 10 mm. or more. Under such conditions the method is satisfactory.

McCord[9] demonstrated that the isolated organs except the heart respond almost without fatigue to repeated injections of pituitary extracts, the effect in nearly every case being markedly constricting on the muscle walls. The heart was depressed, and in a very large majority of the experiments there resulted a decreased rate and amplitude. These results seem to indicate that the pressor effect on the vessels is fairly constant, and discredit any theory of a pressor principle distinct from a depressor principle.

This is corroborated by Roth[6] in the statement: "By the blood-pressure method, a comparison could easily be made between Sample 1 (Pituitrin, P. D. & Co.) and Sample 3 (Infundin), since the pressor response was little diminished even after hours when weak dilutions were used."

Roth further states, however, "On the other hand, it was very difficult to compare Samples 6 (Hypophysin) with Sample 1 (Pituitrin, P. D. & Co.), and impossible to compare Sample 8 (Pituitary Ext. Schering) with Sample 1." This statement has not been verified because if each is injected in sufficient quantity to give equal heights of blood pressure, the activities are certainly in inverse ratio to the dilutions used. If a preparation fails to raise the blood pressure, but with a dose 7½ times as large as another sample has an equal effect on the isolated uterus, who can say that the latter and not the former is the true index to its therapeutic value? If an extract is so poorly prepared that a large quantity is needed to raise the blood pressure measurably, deleterious substances may be present in such a proportion as to obscure the pressor effect. However, would not these other substances have an equally obscuring effect on the oxytocic test?

The foregoing statements regarding the objections raised against the blood-pressure method are not intended to be taken as refuting them but to explain why the authors do not consider

them so vitally important and to lay the facts open for general consideration. The fact that the oxytocic test and the comparison with histamine as a standard are now incorporated in the 9th Revision of the United States Pharmacopœia would indicate that these objections have been either answered or overlooked—possibly the latter, since those most interested in the subject were ignorant of this contemplated step until it was accomplished.

The isolated uterus test, while in some respects logical, is not in its present form practicable to be used as a commercial method for the final standardizing of pituitary extracts. It is a good qualitative test but lacks the accuracy and dependability necessary for commercial quantitative standardization.

While apparently simple both in technic and in the apparatus needed, experience has proved that in the former, at least, it is quite the contrary; the large number of pigs that are not adapted to the test, the slow and unsatisfactory preliminary standardization of the uterus, and the inaccuracy of the test when applied to weak extracts are objectionable features as great as, if not greater than, the objections advanced against the blood-pressure method.

In attempting to apply this method there were weeks at a time when some unfavorable conditions prevented our obtaining results allowing any degree of comparison. Compare also Dale and Laidlaw (*loc. cit.*).

In some cases the uterus is so sensitive as to respond beyond the limits of measurement to minute quantities of an extract. Weighting the lever to overcome part of this sensitiveness often results in killing the sensitiveness even when this is done with the greatest care.

On some occasions (and this is the most frequent cause for rejecting a specimen) it is impossible to obtain two equal consecutive contractions of the uterus from equal amounts of the same extract.

At times the return to normal after a contraction is so slow that whether one waits for spontaneous relaxation or attempts to counterbalance the contraction with weights or bring about relaxation by means of an adrenalin solution, the sensitiveness of the specimen is lost.

That the difficulties of the test have been discovered by every

investigator is evidenced by the fact that there is little agreement among them in the description of the technic employed. Guggenheim claims that the rat is free from some of the objections charged to the guinea-pig. Roth claims that the uterus of a virgin guinea-pig is better than that from dog, cat or mouse. He also claims that for only about two hours can a uterus specimen be used for this work, while Fühner could use the same strip for a week.

The latest report on this point is that of Fenger, which appeared since writing this article,[10] who states that "The selection of a suitable uterus is not always an easy matter. Often three or four pigs have to be killed before a satisfactory strip is obtained. The individual sensitiveness towards pituitary and histamine varies considerably. It is, consequently, by comparing several tracings on different days that a fairly correct conclusion in regard to actual strength of a certain solution can be drawn. It would be advisable to obtain a standard which resembles more closely the active principle of the posterior lobe than does histamine."

With the strictest attention to details some of the objections have been minimized, but when the test cannot be made more accurate than merely to distinguish between dilutions, 1-1000, 1-2000 and 1-3000 [the middle one being considered equal to standard and the others credited with respectively greater and less contractions than standard (see reference 6)], its accuracy leaves much to be desired.

While apparently the oxytocic test on the uterus muscle is the more logical one for standardizing pituitary extracts intended for obstetrical use, one cannot but question whether it more certainly indicates oxytocic activity than does the blood-pressure test. Several substances such as blood serum, beef bouillon, peptone, egg white, putrid meat extracts, act on the uterus muscle without having any recognized oxytocic value. [11, 12] In the case of pituitary extract, is it more than merely a coincidence that it acts on the excised uterus muscle and also on the gravid uterus? Agents with oxytocic value are, without exception, those which react on smooth muscular tissue; the arterioles and small veins, as well as the uterus, belong to this class of muscle. It is the action of pituitary extracts on these unstriated muscles which

brings about increased blood pressure. This seems, therefore, to be a logical test reaction. In this connection another point should be given due consideration—the increasing use of pituitary extract for its blood-pressure effect, particularly during or after surgical operations. By its use the systemic pressure is increased while the pressure in the pulmonary circuit is lowered—a condition described by Wiggers[13] as "a fortunate combination of actions." Shall we apply the oxytocic test exclusively to a substance so valuable for its pressor effects?

From Roth's results[6] we tabulate the following comparisons of pressor and uterine values:

	Values.	
SAMPLE.	PRESSOR.	UTERINE.
1	15	7.5
6	1	1
8	?	1

On the face of this report it would seem possible for the obstetrician to determine whether Sample 1 is 15 times or only 7½ times as active as Sample 6. More striking still is the discrepancy in the case of Sample 8, of which Roth injected 250 times as much as of Sample 1 without an equal rise in blood pressure, while the uterus test would indicate that it was not devoid of activity. This again is contrary to the statements of Dale and Laidlaw[3] that the pressor and oxytocic effects seem to go hand in hand, an observation made also by Frankl-Hochwart and Fröhlich[14] and repeatedly confirmed in our own work.

The problem seems to be one not for the pharmacologist alone, but for the clinician as well.

While we are not condemning as useless the uterus test for standardizing pituitary extracts, we are not willing to subscribe to the popular opinion that this test alone is deserving of recognition, that it has greater accuracy as a standard method of assay than the pressor test, or that the latter is not a measure of oxytocic activity.

We conclude, therefore:

First, that neither method in its present form is ideal as a means of standardizing pituitary extracts.

Second, that most specimens of the isolated guinea-pig uterus are sensitive to the action of these extracts, some are exquisitely sensitive, but few are sufficiently uniform in the reaction to be used for accurate standardization purposes.

Third, that the pressor test is a fairly accurate measure of pituitary activity, is not an illogical indicator of oxytocic value, and is free from some of the most objectionable features of the uterine method.

We regret our inability at the present time to add anything to the refinement of either method, but suggest, first, that the possibilities of the pressor test be further developed since it has been found capable in our hands of recognizing differences in pressor activity of less than 10 per cent; second, that chemists, pharmacologists, and clinicians work together in an effort to determine whether pressor and oxytocic values depend on the same active principle.

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THE VARIABILITY OF STROPHANTHIN WITH PARTICULAR REFERENCE TO OUABAIN.*

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The importance of Strophanthus in therapeutics has led to much original investigation of its activity both chemical and physiological. Although it has been many years since Fraser[1] first obtained an active principle from strophanthus seed which he called Strophanthin, the preparation of a chemically pure Strophanthin, *i.e.*, one of constant chemical and physiological properties, has not been consistently accomplished. It is true that Fraser's method has been improved and new methods worked out by which a more active strophanthin has been obtained, but commercial preparations from the same kind of seed still show a surprising lack of uniformity in physiological activity and in chemical purity as well.[3] A large part of this variability can no doubt be attributed to the difficulty encountered in obtaining seed of one particular kind. It is now known that there are more than 20 different species of Strophanthus Seed on the market[2] and it is a relatively common occurrence to find upon close examination that a given lot of seed purchased as *Kombe* contains some seed of other varieties.

There are only three different strophanthins (*Kombe-strophanthin, hispidus-strophanthin* and *gratus-strophanthin* or *ouabain*) of which any chemistry or pharmacology is known, and these differ considerably in the degree of their physiological activity. Consequently, if the strophanthin is not obtained from seeds of the same species, its activity will be greater or less than the average for that kind of seed.

Another reason for the variability of strophanthin lies in the fact that some species of seed such as *Kombe* contain a crystalline and an amorphous acid strophanthin which are closely related but which differ markedly in quantitative physiological activity. If the product is very carefully crystallized and labeled "Crystal-

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line Strophanthin," this cause of non-uniformity should disappear, but it is sometimes difficult to obtain a definite separation of the crystalline from the amorphous variety.

The chemistry and pharmacology of the strophanthins derived from *Strophanthus Kombe* have been carefully studied, but Ouabain, the strophanthin from *Strophanthus Gratus,* which seems to be identical with the Ouabain from some species of *Acocanthera,* has not been so thoroughly investigated. It is known to be a nicely crystalline body which may contain variable amounts of water of crystallization, but, since it does not yield a crystalline strophanthidin as *Kombe strophanthin* does, the study of its chemistry is under a disadvantage and the identity of the glucoside is to that extent uncertain. The fact that different samples of Ouabain may contain variable amounts of water of crystallization is a source of serious disagreement in the activity of various samples.

In the quantitative testing of heart tonics of the digitalis series such as Digitalis, Strophanthus, and Squill, physiological methods have been found to be the most feasible. A number of methods have been proposed, but most of them agree on one thing, namely, that the frog is best suited for use as the test animal. The new Pharmacopœia (ninth revision) recommends that heart tonics be assayed by the use of one of these frog methods, the "One-Hour Method."[6] This method consists in brief of determining the smallest dose of the heart tonic preparation per unit of weight of the test animal which will just stop the heart of a frog in systole one hour after the dose is administered, and is, therefore, the minimum systolic dose per gram weight of frog for one hour. This dose must be compared with that of a standard preparation to be tested at the same time in order to take into consideration the variation in resistance of the frogs from season to season and to a less degree from day to day.

The standard recommended for use is Ouabain, the strophanthin obtained from *Strophanthus Gratus.* In the selection of a standard, the chief consideration should be the purity of the preparation, its stability, its universal availability and its qualitative and quantitative activity. It is true that Ouabain is a crystalline compound, a fact which should point to its purity and stability. However, different samples contain variable amounts

of water of crystallization so that the activity is not uniform. Even *Kombe Strophanthin,* which has been more thoroughly studied chemically and which seems to be a constant chemical compound, is not found to be of uniform physiological activity when various samples on the market are purchased and tested. The following tests of samples of *Kombe Strophanthin* made during the last few years serve to show the variable activity of the commercial preparation:

TABLE I.

Date	Activity M. F. D. Sample	M. F. D. Std.	Percent of Std.
(1) May 8, 1906	.00000050	.00016	192
(2) November 10, 1906	.00000040	.00016	240
(3) February 22, 1907	.00000045	.00013	173
(4) April 4, 1907	.00000050	.00015	180
(5) May 18, 1907	.00000060	.00016	160
(6) July 7, 1907	.00000050	.00016	192
(7) June 10, 1908	.00000050	.00015	180
(8) October 9, 1909	.00000070	.00011	94
(9) July 18, 1911	.00000040	.00009	135
(10) December 7, 1911	.00000100	.00013	78
(11) December 11, 1911	.00000100	.00013	78
(12) January 16, 1912	.00000086	.00010	67
(13) January 13, 1913	.00000055	.00011	120
(14) October 8, 1913	.00000045	.00011	145
(15) April 10, 1914	.00000065	.00011	102
(16) August 21, 1914	.00000055	.00011	120
(17) September 25, 1914	.00000070	.00011	93
(18) April 28, 1915	.00000055	.00011	120
(19) June 15, 1915	.00000060	.00012	120
(20) October 9, 1915	.00000075	.00012	96
(21) March 4, 1916	.00000055	.00010	110
(22) August 8, 1916	.00000055	.00010	110

From this table it can be seen that the least active sample was 67 per cent of standard, while the most active was 240 per cent or approximately 3.5 times as active. The ten samples tested since January 1, 1913, have shown a much greater degree of uniformity in activity. In this later period the most active sample was but 1.5 times as strong as the least active sample. It has also been shown experimentally that a crystalline Strophanthin can be prepared from *Kombe* seed (the official drug), which is constant in chemical composition and physiological activity.[3] In order to do this, great care must be exercised in the selection of

the seed and in following a definite method of preparation and purification.*

The facts favoring the selection of Ouabain as a standard for the testing of heart tonics of the digitalis series might be advanced as follows:

First, that Ouabain is the only active principle of *Strophanthus Gratus* whereas the *Kombe* seed contains two or more.

Second, that it is a nicely crystalline body.

Third, that it is the most active strophanthin yet obtained.

Fourth, that its absorption is comparatively rapid with very slight if any tendency to cumulative action.

These advantages appear to be more than offset by the disadvantages previously mentioned, such as the variable content of water of crystallization and the consequent variation in physiological activity of samples of Ouabain, and the fact that its chemistry and pharmacology have not been so thoroughly investigated as has that of *Kombe Strophanthin.* The fact that *Kombe* seed rather than *Gratus* seed is official should make the crystalline *Kombe Strophanthin* the logical choice as a standard. The greater activity of Ouabain can scarcely be considered of any advantage and the *Kombe Strophanthin* is readily absorbed, even though not quite so rapidly as Ouabain. In fact the greater toxicity of Ouabain may be due in part to the greater rapidity with which it is absorbed.

In order to determine whether the physiological activity of Ouabain varies, three samples were purchased at different times

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**Method of Preparation.*—1.5 Kg. ground, fat-free Strophanthus Seed was percolated with 12 liters 70-per-cent alcohol and the percolate was distilled off *in vacuo* until about 1 liter fluid remained. To this fluid sufficient lead subacetate solution (Liquor plumbi subacetatis, U. S. P.) was added, to obtain an easily filtering mixture. The filtrate is a clear yellow fluid. The excess of lead was removed by hydrogen sulphide and the clear filtrate was evaporated at 40°-45° with constant stirring. Until the fluid becomes concentrated it is important that it be kept alcoholic by frequent addition of a little alcohol. When the fluid has become a thin extract, the alcohol must be evaporated as much as possible. It will then crystallize readily. The crystals are separated on a hardened filter of large surface by suction. The recrystallization is made in the following manner to avoid conversion into the amorphous body: The crystals are dissolved by placing them in a dish with a small amount of 9-per-cent alcohol and heating to 40°-50° and stirring occasionally. After having filtered the solution, the alcohol is now evaporated to a thick extract at this temperature and water is added until a thin extract is obtained. With constant slow stirring with a motor, the extract is again evaporated to remove the rest of the alcohol. The extract will then crystallize readily.

Crystalline strophanthin as we have shown can be obtained without any chemical purification from the alcoholic extract. It is thus possible to determine whether a certain chemical method of preparation of strophanthin from Strophanthus Kombe seed gives a yield of naturally occurring crystalline strophanthin or of a derivative of strophanthin.[2]

and submitted to physiological assay. These assays were made by two methods and at several different times. The following table shows the results obtained in the various tests of these three samples of Ouabain:

TABLE II.

TESTS OF THREE SAMPLES OF OUABAIN BY TWO METHODS.

M.L.D. METHOD.

SAMPLE "A," 1 OZ.

M.L.D.	.00000045	June 21, 1915. (Std. .00013)
M.L.D.	.00000035	April 29, 1916. (Std. .00011)
M.L.D.	.00000030	August 21, 1916. (Std. .00011)
Aver.	215500 H.T.U. per Gm.	

ONE-HOUR METHOD.

SAMPLE "A," 1 OZ.

M.S.D.	.00000080	June 21, 1915.
M.S.D.	.00000080	February 15, 1916.
M.S.D.	.00000080	March 3, 1916.
M.S.D.	.00000110	April 12, 1916.
M.S.D.	.00000080	May 4, 1916.
M.S.D.	.00000090	May 9, 1916.
M.S.D.	.00000090	May 9, 1916.
M.S.D.	.00000080	August 16, 1916.
Aver. M.S.D.	.00000086	

M.L.D. METHOD.

SAMPLE "B," 5 GM.

M.L.D.	.00000045	June 21, 1915. (Std. .00013)
M.L.D.	.00000045	August 25, 1916. (Std. .00012)
Aver.	185200 H.T.U. per Gm.	

ONE-HOUR METHOD.

SAMPLE "B," 5 GM.

M.S.D.	.00000065	June 21, 1915.
M.S.D.	.00000080	February 15, 1916.
M.S.D.	.00000090	August 21, 1916.
M.S.D.	.00000080	August 16, 1916.
Aver. M.S.D.	.000000787	

M.L.D. METHOD.

SAMPLE "C."

M.L.D.	.00000040	April 22, 1916. (Std. .00011)
M.L.D.	.00000035	April 29, 1916. (Std. .00011)
M.L.D.	.00000040	August 25, 1916. (Std. .00012)
Aver.	.197600 H.T.U. per Gm.	

TABLE II.—*Continued.*

ONE-HOUR METHOD.

SAMPLE "C."

M.S.D.	.0000010	April 7, 1916.
M.S.D.	.0000009	March 21, 1916.
M.S.D.	.0000010	May 4, 1916.
M.S.D.	.0000010	May 9, 1916.
M.S.D.	.0000008	August 22, 1916.
Aver. M.S.D.	.00000094	

From this table it can be seen, first, that the three samples are more nearly uniform when tested by the "M.L.D. method"[7] than when tested by the "one-hour method," and, second, that the average M.S.D. for any of the samples is much larger than that chosen as the standard for the "one-hour method" by the Pharmacopœial Revision Committee.

In order to bring out these points more clearly, the results should be considered from a number of view-points. By the "M.L.D. method" the variation in activity in the three samples is 14 per cent, while the average activity of the three is almost exactly double that of the average *Kombe Strophanthin*. However, by the "one-hour method," which is the method officially recommended, the variation in activity in the three samples is somewhat greater, but the greatest discrepancy is the variation of each of the samples from average M.S.D. (.00000050 Gm. per Gm. wt. of frog) which is officially proposed for comparison. Since the M.S.D. of these three samples was determined in the different seasons and an average taken, and since in no test was the dose found to be as small as that mentioned in the Pharmacopœia as being standard for Ouabain, it is very evident that all of these samples of Ouabain are considerably below standard. In fact, sample "C," which was expressly obtained for use as a standard, is but 53 per cent as active as it should be. Sample "B," which was the most active according to the "one-hour method," is only 63 per cent as active as the standard proposed.

It is difficult to believe that three different samples obtained at different times from reliable sources should have an M.S.D. so much greater than the average proposed by the revision committee. The three samples were assayed so many times in different seasons and with such universally high results that any experimental error must have been eliminated. It seems quite

probable therefore that the M.S.D. chosen by the committee is smaller than it should be since it is generally admitted that Ouabain is approximately twice as active as crystalline *Kombe Strophanthin* [4,5] and the M.S.D. of the latter preparation has been found experimentally to be approximately .0000015 Gm. per Gm. wt. of frog. The logical deduction from the foregoing data is that the average M.S.D. of active Ouabain is about .00000075 Gm. per Gm. body weight, which is approximately the average found in our work.

From the data submitted it seems reasonable to conclude that the variation in activity of different samples of Ouabain is too great to admit of its use as a satisfactory standard in the testing of heart tonic preparations by the "one-hour method," and that the average minimum systolic dose obtained in a number of assays of three different samples is much larger than that proposed by the committee.

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REPRINTS OF PUBLICATIONS FROM THE RESEARCH LABORATORY, PARKE, DAVIS & CO., DETROIT, MICH.

The present system of collecting reprints of articles published from the Research Laboratory was begun in 1912. Reprints of the following articles published subsequent to that time are available and will be sent upon request. The publications marked (*) are no longer available.

1. On the Administration of Diphtheria Toxin in a Collodion Sac. By E. C. L. Miller. (*Journal of Infectious Diseases,* Vol. 8, January, 1911, pp. 50-65.)

2. A Further Contribution to Our Knowledge of Insecticides—Fumigants. By Chas. T. McClintock, H. C. Hamilton and F. B. Lowe. (*Journal of the American Public Health Association,* Vol. 1, April, 1911, pp. 227-238.)

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THE GENUS HIPPOCHAETE IN NORTH AMERICA, NORTH OF MEXICO.

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My attention was specifically drawn to these plants through the monograph of the genus *Equisetum* by Mr. A. A. Eaton, which appeared serially in the Fern Bulletin, and in which the exclusion of *E. lævigatum* and *E. robustum* from Michigan was so opposed to my field studies of these species, as I understood them, that I concluded to give the subject further and more careful attention in order to confirm my earlier views or to reject them. Mr. Eaton, it is true, credited *E. robustum* to Sarnia, Michigan, but Sarnia is in Ontario, Canada. These two species are unquestionably found in Michigan.

The following notes are based primarily on field studies, supplemented by studies of material distributed by Eaton and of that in the herbarium of Parke, Davis & Co.

Mr. Eaton said there were no true varieties, with perhaps the exception of *E. arvense* var. *boreale,* in the genus *Equisetum;* and then listed a large number, many of them new, but specifically made no claims for their constancy. A goodly number of these so-called varieties are based entirely on an injury to the individual plant, and therefore are no more deserving, from a systematic point of view, of a special name and the dignity which is always conferred by the elevation of a form to one of the named nomenclatorial categories, than is, in the higher plants, a willow stump that has sent out innumerable branches to prolong its existence; or if it pleases you to have the subject matter brought a little nearer home, than is a man with an artificial limb to be ranked as a new species in the genus *Homo.* All these so-called varieties may be produced at will from the same evergreen stalk. The apex of a normal evergreen stem may be broken off and labeled under its specific name; the following year another section, now with long branches, may be broken off and labeled var. *ramigerum;* the next year what is left of the stem may be bearing the short spiciform branches of the var. *ramosum;* and the next season will see numerous stems surrounding the base of the

old one, whence we have the var. *cæspitosum*. To apply names of specific or other rank to such forms is the climax of folly. On the other hand, when a species is normally unbranched, if a form of it is found that constantly produces branches naturally, *i.e.*, not due to an injury, such a form is a normal variety; such a form can not be found on the same rhizome with another of different character, while all the so-called varieties due to an injury may be found along with the normal stem emanating from the same rhizome and sometimes two or more such forms may be found on the same crown! I have observed several forms due to an injury, that do not come within the description of any variety enumerated by Mr. Eaton; but they are as much deserving of recognition as any such that he has named.

With special reference to the bast and green parenchyma, there are two very distinct types of anatomy in the genus *Hippochæte:* one with abundant vallecular bast completely cutting the green parenchyma while the carinal bast is slight and does not completely cut the parenchyma, thus dividing it into Y-shaped blocks; the other is just the reverse of this, the vallecular bast, being of small amount, does not divide the parenchyma, while the carinal is plentiful and does divide it, or nearly so, thus splitting the green parenchyma into blocks shaped somewhat like a carpenter's drawing knife. *H. lævigata* best exemplifies the former, while *H. prealta* is typical of the latter. Similar to *H. lævigata* are *H. variegata* and *H. hyemalis* var. *alaskana*. Like *H. prealta* are *H. prealta* var. *affinis, H. hyemalis* var. *californica, H. scirpoides,* and the European *H. hyemalis*. The other species and varieties are either intermediate in character or they combine both types in greater or less degree. In the intermediate forms the parenchyma is continuous under both the vallecular and carinal basts, the outer surface being even, or more or less indented but not divided by the basts. When the two types are combined the Y-shaped parenchyma may alternate with the other type, or the parenchyma may be divided by both the vallecular and carinal basts splitting it up into irregularly triangular blocks. The central cavity is also variable, ranging from obsoleteness in *H. scirpoides* to 4/5 the total diameter of the stem in *H. prealta* and *H. lævigata*. It has been suggested that all variations from the two types above mentioned, in the anatomy of some forms,

are due to hybridization. This may be so, but it has yet to be proved by exact laboratory work. On the other hand, varieties combining these intermediate or variable anatomical characters, such as *H. variegata* var. *anceps* and *H. hyemalis* var. *Jesupi* are sometimes found where the supposed parents have never been detected; the former on Parkedale Farm and the latter on Belle Isle, an island in the Detroit River, which has 5 or 6 miles of shore line with the nearest point of mainland ½ a mile away. It is the only *Hippochæte* that has ever been detected on the island.

In certain California plants Mr. Eaton ascribes to **Hippochaete ramosissima** (Desf.) comb. nov. (*Equisetum ramosissimum* Desf. Fl. Atl. 2: 398. 1800) an anatomy like that of *H. lævigata.* According to Luerssen the parenchyma of this species is continuous, thus being intermediate but more similar to that of **Hippochaete hyemalis** (L.) comb. nov. (*Equisetum hyemale* L. Sp. Pl. 1062. 1753) than to *H. lævigata.* If Luerssen is correct in regard to the anatomy of *H. ramosissima,* then the Californian plants are not of that species and it should be excluded from the American flora. Notwithstanding that Sadebeck gives it a range on the American continents from 49° north latitude to 30° south latitude, it is highly improbable that it occurs north of central Mexico. Judging from the anatomy, as described by Mr. Eaton, the plants he referred to *H. ramosissima* should be referred to some form of *H. lævigata,* probably to var. *Funstoni.*

From an intimate study of these plants in the field, extending over many years, I have become thoroughly imbued with the conviction that they constitute a valid genus distinct from *Equisetum.* The habit and other characters by which *Hippochæte* differs from *Equisetum* are as constant and of as much significance as those which separate *Aster* from *Solidago* in the higher plants, and many other closely allied genera which could just as readily be named. Generally speaking their differences may thus be expressed.

Stems annual, often dimorphous, the sterile always with regular verticils of acutely angled branches at the nodes; spikes rounded at apex; stomata scattered. EQUISETUM.

Stems generally evergreen, not dimorphous, usually simple; branches when present, similar to the stem; spikes usually apiculate; stomata in regular rows. HIPPOCHÆTE.

Of the genus *Equisetum* (Tourn.) L., the type species is *E. arvense;* of *Hippochæte* Milde, *H. hyemalis.* I have not been able to verify Milde's authorship of the genus, but according to Ascherson and Graebner, Milde published it in the Botanische Zeitung for 1865, p. 297. The genus is readily subdivided into two sections. The species with evergreen stems and apiculate spikes form a group that may be known as section EUHIPPOCHÆTE; those with annual stems and generally obtuse spikes may be known as section AMBIGUA.

The chief character separating the sections is the duration of the stem; those separating the species are to be found in the ridges and sheaths, whether concave and biangulate, or convex and banded, campanulate or cylindrical. Following out these lines of differentiation, the species will be assembled in a more natural grouping than by any other method.

KEY TO THE SPECIES.

Stems evergreen, spikes apiculate (EUHIPPOCHÆTE).
- Ridges concave, biangulate.
  - Ridges narrowly concave, numerous.
    - Sheaths cylindrical, tight.
      - Centrum ¼ the diameter of the stem, teeth deciduous or more or less persistent. — *H. hyemalis* var. *californica.*
      - Centrum, ½ the diameter of the stem; teeth and awns persistent. — *H. hyemalis* var. *Jesupi.*
      - Centrum ⅓ the diameter of the stem; awns deciduous. — *H. hyemalis* var. *alaskana.*
    - Sheaths campanulate, loose.
      - Centrum ⅓ the diameter of the stem; teeth persistent, awns deciduous. — *H. variegata.*
      - Centrum 1/6 the diameter of the stem to obsoleteness. — *H. variegata* var. *anceps.*
  - Ridges broadly and deeply concave, these and the teeth three. — *H. scirpoides.*
- Ridges rounded, not biangulate.
  - Sheaths cylindrical, tight.
    - Stems simple.
      - Sheaths broader than long, teeth persistent. — *H. prealta.*
      - Sheaths longer than broad, teeth caducous. — *H. prealta* var. *affinis.*
    - Stems normally branched; branches spike bearing. — *H. prealta* var. *Suksdorfi.*
  - Sheaths more or less ampliate and loose, but not campanulate.
    - Teeth caducous. — *H. prealta* var. *intermedia.*
    - Teeth deciduous or persistent. — *H. prealta* var. *scabrella.*

Stems annual, spikes obtuse or apiculate, ridges rounded (AMBIGUA).	
Sheaths cylindrical, tight; teeth persistent.	*H. Nelsoni.*
Sheaths campanulate and loose; teeth caducous.	
Stems smooth to the touch.	*H. lævigata.*
Stems rough, simple.	
Bases of teeth straight.	*H. lævigata* var. *Eatonii.*
Bases of teeth, incurved.	*H. lævigata* var. *Funstoni.*
Stem rough, branched.	*H. lævigata* var. *polystachya.*

HIPPOCHÆTE HYEMALIS var. **californica** (Milde) comb. nov.

Equisetum hyemale var. *californicum* Milde; A. A. Eaton, Fern Bull. **11**: 113. 1903, as to the biangulate plants only.

Equisetum hiemale var. *Doellii* Milde; A. A. Eaton, Fern Bull. **11**: 114. 1903. Not Milde, 1863.

Typical *H. hyemalis* with caducous teeth has never been collected in America. The Pacific coast plants differ in having the teeth deciduous or more or less persistent. The plants referred by Mr. Eaton to the var. *Doellii* Milde cannot belong there as that European variety has the centrum only one-fourth or one-third the diameter of the stem, while the British Columbia plants have a centrum four-fifths the diameter of the stem and the teeth are not wholly persistent as described, at least on specimens distributed which show them to be generally deciduous. California to British Columbia.

HIPPOCHÆTE HYEMALIS var. **Jesupi** (A. A. Eaton) comb. nov.

Equisetum variegatum var. *Jesupi* A. A. Eaton, Fern Bull. **12**: 24. 1904.

This variety has some of the characters of *H. variegata,* towards which it trends. The teeth have long, generally persistent awns and the anatomy is variable, sometimes of one type, sometimes of the other. The size and aspect are intermediate, but the tight, cylindrical sheaths place it with *H. hyemalis* rather than with *H. variegata.*

Belle Isle, Mich., *Farwell 211a,* June 4, 1895; Rochester, Mich., *Farwell 211b,* July 4, 1896. Mr. C. K. Dodge has collected it at Port Huron, Mich. Its general distribution is from Illinois to Connecticut, northward into Canada.

HIPPOCHÆTE HYEMALIS var. **alaskana** (A. A. Eaton) comb. nov.

Equisetum variegatum var. *alaskanum* A. A. Eaton, Fern Bull. 12: 39. 1904.

Somewhat similar to the last variety but larger, yet with a relatively smaller centrum, awns deciduous, and anatomy much like that of *H. variegata*. The cylindrical, tight sheaths place it here rather than with the species just named. In dried plants the sheaths are liable to be slightly ampliated. Washington to Alaska.

Hippochaete variegata (Schleich.) comb. nov.

Equisetum variegatum Schleich. Cat. Helvet. 27. 1807.

This species, in its typical form, is common on sandy or gravelly shores on the Keweenaw Peninsula, a tongue of land 60 miles in length, stretching northeasterly into Lake Superior. It is associated with *H. lævigata* and *Equisetum limosum*. I have seen no indications that either this or the variety *anceps* is injured by frost in this State, and they are certainly evergreen.

Keweenaw Peninsula, Mich., *Farwell 211*, May 30, 1885. This species may be looked for north of 42°. It prefers the borders of cold streams and ponds.

HIPPOCHÆTE VARIEGATA var. **anceps** (Milde) comb. nov.

Equisetum variegatum var. *anceps* Milde, Ann. Mus. Lugd. Bat. 1: 71. 1863.

This variety is intermediate in aspect between the species and *H. scirpoides* and approaches the latter in habit. The stems are evergreen, 30 cm. or less in height by 1 mm. or less in diameter, and are massed in a mat-like growth. The centrum is from obsolete to one-sixth the diameter of the stem and does not exceed the vallecular cavity; the anatomy is variable. The ridges and leaves are 4-8, the teeth persistent, and the awns deciduous. The branches have the same number of ridges and leaves as the stems, or fewer, and in some instances they have five leaves while the stem has four; the only instance, so far as I am aware, where a branch has more ridges and leaves than the stem from which it springs. It grows on grassy borders of marl under willow thickets associated with moss and sedges. Parkedale Farm, Mich., *Farwell 2921*, July 28, 1912.

Hippochaete scirpoides (Michx.) comb. nov.

Equisetum scirpoides Michx. Fl. Bor.-Am. **2**: 281. 1803.

Our smallest species, forming dense mats along old logs and stumps in open fields, cedar swamps, etc. Well characterized by its three leaves and ridges, the latter so deeply and broadly concave that the stem apparently is 6-ridged.

Keweenaw Peninsula, Mich., *Farwell 212,* May 30, 1885. This has much the same range as *H. variegata* but extends about 2° further south.

Hippochaete prealta (Raf.) comb. nov.

Equisetum prealtum Raf. Fl. Ludovic. 13. 1817.

Equisetum robustum A. Br. Am. Jour. Sci. 46, 88. 1843.

Equisetum robustum var. *minus* Engelm. in A. Br. Am. Jour. Sci. 46, 88. 1843.

Equisetum hiemale var. *Drummondi* Milde, Mon. Equit. 593. 1865 (?).

Equisetum hiemale var. *robustum* A. A. Eaton, Fern Bull. **11**: 112. 1903.

Equisetum hiemale var. *californicum* Milde; A. A. Eaton, Fern Bull. **11**: 113. 1903, as to the plants with rounded ridges.

Equisetum hyemale var. *prealtum* (Raf.) Clute, Fern Bull. **16**: 18. 1908.

This is our largest and most widely spread species and probably our most common one. It is well characterized by its cylindrical, tight sheaths, which are as broad as long, or sometimes a little broader or narrower than long, its persistent teeth, and very numerous, rounded ridges. It may be found on sand banks, in poor soil, in swamps and bogs; generally near water. Mr. C. K. Dodge has collected it near Port Huron, Mich., and near Sarnia, Ontario.

Keweenaw Peninsula, Mich., *Farwell 209½,* May 30, 1885; *572,* Aug. 29, 1887. Rochester, Mich., *Farwell 2975,* Aug. 4, 1912; *3696* and *3710,* June 28, 1914; *3722,* July 19, 1914; *3922* and *3927,* October 25, 1914. Parkedale Farm, Mich., *Farwell 3988½,* June 20, 1915. May be found from the Atlantic to the Pacific, but rare east of the Mississippi basin.

HIPPOCHÆTE PREALTA var. **affinis** (Engelm.) comb. nov.

Equisetum robustum var. *affine* Engelm. in A. Br. Am. Jour. Sci. **46**: 88. 1843.

Equisetum hiemale var. *pumilum* A. A. Eaton, Fern Bull. **11**: 109. 1903.

Equisetum hiemale var. *affine* (Engelm.) A. A. Eaton, Fern Bull. **11**: 111. 1903.

Differs from the species in its longer sheaths which are ⅓-2 times as long as broad, and in the teeth, which are caducous. Where this variety and the species overlap they intergrade and pass insensibly one into the other, indicating that there is but one species, although the extremes seem distinct enough.

Keweenaw Peninsula, Mich., *Farwell 209,* May 30, 1885. Palmer Park, Mich., *Farwell 209a,* July 15, 1902. Rochester, Mich., *Farwell 3694* and *3695,* June 28, 1914; *3925, 3926,* October 25, 1914; *3929½,* October 29, 1914. Parkedale Farm, Mich., *Farwell 3922½,* October 25, 1914; *3984½,* June 20, 1915. From the Atlantic to the Pacific, but most common east of the Mississippi River.

HIPPOCHÆTE PREALTA var. **Suksdorfi** (A. A. Eaton) comb. nov.

Equisetum hiemale var. *Suksdorfi* A. A. Eaton, Fern Bull. **11**: 110. 1903.

Somewhat similar to the variety *affinis* but has whorled branches from the upper nodes, which are spike-bearing simultaneously with the central stem; Mr. Eaton described it as with the anatomy of *E. hiemale,* but material distributed by him shows the green parenchyma cut by the vallecular bast which, with the branching habit, indicates a tendency toward *H. lævigata,* while the rosulæ in the groove show a trend toward *H. hyemalis* var. *californica.* Collected by W. N. Suksdorf, September 3, 1902, at Bingen, Washington.

HIPPOCHÆTE PREALTA var. **intermedia** (A. A. Eaton) n. comb.

Equisetum hiemale var. *intermedium* A. A. Eaton, Fern Bull. **10**: 120. 1902, pp., and in Gray's New Manual, 53. 1908.

The sheaths are ampliated but not campanulate, and the teeth are caducous. Described by Mr. Eaton as with the anatomy of *Equisetum hiemale,* but material distributed by him shows a vari-

able anatomy, sometimes that of *H. prealta*, sometimes that of *H. lævigata*. Collected at Port Huron, Michigan, by Mr. C. K. Dodge. It has been found in various localities from the Atlantic to the Pacific and may be looked for whenever *H. lævigata* and *H. prealta* var. *affinis* may be found in close association.

Hippochæte prealta var. **scabrella** (Engelm?) comb. nov.

? *Equisetum lævigatum* var. *scabrellum* Engelm. in A. Br. Am. Jour. Sci. **46**: 87. 1843.

? *Equisetum hiemale* var. *texanum* Milde; A. A. Eaton, Fern Bull. **11**: 108. 1903.

Similar to the preceding variety, but the sheaths are proportionably broader and the teeth deciduous or persistent, indicating a cross between *H. lævigata* and *H. prealta*, if these intermediate forms are to be considered as the result of hybridization. I do not know if this is Engelmann's variety or not, but it agrees in every particular with Eaton's description of *Equisetum laevigatum* var. *scabrellum* in Fern Bull. **11**: 42. 1903. The stems of the season have the general aspect that *H. lævigatum* would have if its sheaths had persistent teeth; the stems of the preceding year have the general markings of *H. prealta*. The anatomy is now of the one species, now of the other.
Rochester, Mich., *Farwell 3712½*, July 4, 1914.

Hippochaete laevigata (A. Br.) comb. nov.

Equisetum lævigatum A. Br. Am. Jour. Sci. **46**: 87. 1843.

This species in its typical form is well characterized by its simple or branched, annual, stems, which are smooth, at least to the touch, its rounded spikes, and campanulate sheaths with caducous teeth. Those varieties which are intermediate between this and othr species generally will have rough stems and spikes that are either obtuse or apiculate. It may be found in clear sand or gravel, or a similar soil covered with a sparse growth of grass and other vegetation and generally not far from water.

It may be found in colonies by itself, or it may be associated with *H. prealta* and its variety *affinis*, *H. variegata*, and *Equisetum limosum*. The vallecular bast divides the green parenchyma into y-shaped divisions. Eaton restricted this species east of the Mississippi to Ohio, Indiana, Illinois, and Wisconsin; but I have found it in southeastern Michigan, where it is com-

mon, and on the Keweenaw Peninsula, where it can not be said to be scarce. Probably it is to be found throughout the State. The annual stems begin their growth about the first of May, are fruiting in June, and perish in July or August. New stems are appearing continuously until the middle of the summer, but all have perished before winter has set in. It may be noted here that growth in the evergreen species begins, in Michigan, about the middle of May and continues through the summer.

Keweenaw Peninsula, Mich., *Farwell 3994½,* June 29, 1915. Algonac, Mich., *Farwell 3640, 3684½, 3685,* June 21, 1914. Detroit, Mich., *Farwell 210c,* June 24, 1902. Rochester, Mich., *Farwell 210c,* July 4, 1896; *3721½,* July 19, 1914. Stony Creek, Mich., *Farwell 3438½,* June 8, 1913. Parkedale Farm, Mich., *Farwell 2701,* June 11, 1912; *3677½,* June 11, 1914; *3705,* June 28, 1914. Common west of the Mississippi and in the "Lake States."

HIPPOCHÆTE LÆVIGATA var. **Eatonii** var. nov.

Equisetum hiemale var. *intermedium* A. A. Eaton, Fern Bull. 10: 120. 1902, as to the annual plant.

Externally, this variety can be distinguished from the typical species only by the roughness of the stem and the occasionally apiculate spikes. The anatomy is very variable, sometimes that of the species, sometimes that of *H. prealta* var. *affinis;* now intermediate when the parenchyma is continuous, and now combining both types when both the carinal and vallecular basts divide the green parenchyma, splitting it into irregularly triangular blocks. It may be found alone, associated with *H. lævigata, H. prealta,* or its var. *affinis,* or with all of these. In the original description of *Equisetum hiemale* var. *intermedium,* Eaton included annual and evergreen plants with teeth that were caducous, deciduous, and persistent. In the seventh edition of Gray's Manual he had restricted the variety to the evergreen plant with caducous teeth. This left the evergreen plant with broader sheaths and persistent teeth and the annual plant with caducous teeth without names. For the former I have adopted Engelmann's varietal name of *scabrella;* to the latter I give the varietal name *Eatonii.* The first stems of the season fruit in June and perish in July and August when the later stems are

fruiting and others just coming up. At this time it simulates *H. prealta* var. *intermedia* but is quickly and readily differentiated by its annual stems, which have not the bright green of that variety. Some plants have completely perished before winter sets in, while others in greater or less degree survive the winter, but these parts have perished before the new growth of the season begins in May. Where this variety grows in profusion it is not an uncommon thing to see in March, just after the snow has disappeared, its long stems chalk-white and intact lying flat upon the ground, crossed in all directions. When disturbed, however, they will fall apart and crumble into powder. The stems like those of the species may be single or caespitose, simple or branched, and often four feet in height. I have not seen any with spike-bearing branches.

Wiards Siding, Mich., *Farwell 2159½, 2159⅓*, June 25, 1910. Rochester, Mich., *Farwell 2706½, 2710½*, June 11, 1912; *3643½*, May 26, 1914; *3694½*, June 8, 1914. Algonac, Mich., *Farwell 3640a*, July 26, 1914.

HIPPOCHÆTE LÆVIGATA var. **Funstoni** (A. A. Eaton) comb. nov.

Equisetum Funstoni A. A. Eaton, Fern Bull. **11**: 10-12. 1903 (excluding forma *polystachyum*).

Equisetum lævigatum f. *variegatoides* A. A. Eaton, Fern Bull. **11**: 43. 1903.

? *Equisetum hiemale* var. *herbaceum* A. A. Eaton, Fern Bull. **11**: 108-9. 1903.

Similar to the specific type but very rough, and the bases of the caducous teeth are more strongly incurved. Eaton described the spikes of *E. Funstoni* as "not apiculate as in the rest of the sub-genus." *H. lævigata* has non-apiculate, *i.e.*, rounded, spikes, and *E. Funstoni* as distributed by Eaton has some of the spikes apiculate. The parenchyma is sometimes divided by the vallecular bast, sometimes not. *Equisetum lævigatum* f. *variegatoides* and *Equisetum Funstoni* f. *caespitosum* as distributed by Eaton are to be differentiated by only one character; the stems of the former are prostrate, ascending, or erect, while those of the latter are ascending or erect—a distinction without a difference. Both have prominently white-bordered teeth fading to white through-

out. There seems therefore to be no good reason for keeping *Funstoni* separate from *lævigata.* It has been collected in Wisconsin, Nebraska, Kansas, Wyoming, Utah, and California.

HIPPOCHÆTE LÆVIGATA var. **polystachya** (A. A. Eaton) comb. nov.

Equisetum Funstoni f. *polystachyum* A. A. Eaton, Fern Bull. **11**:12. 1903.

Equisetum lævigatum f. *polystachyum* A. A. Eaton, Fern Bull. **11**:44. 1903.

A form in which the stem and its branches (not due to an injury) are simultaneously spike-bearing.

Hippochaete Nelsoni (A. A. Eaton) comb. nov.

Equisetum variegatum var. *Nelsoni* A. A. Eaton, Fern Bull. **12**:41. 1904.

Intermediate between *H. lævigata* and *H. hyemalis* var. *Jesupi,* but more like the latter in appearance than the former. The rounded ridges and annual stems, however, place it more appropriately in the section Ambigua than in the Euhippochæte. The parenchyma is frequently divided by the vallecular bast but not regularly so. The sheaths in dried plants are liable to be slightly ampliated. It has been collected in New York, Michigan, and Illinois.

FURTHER STUDIES ON HOG CHOLERA WITH REFERENCE TO SPIROCHAETA HYOS.*

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INTRODUCTION. Several reports have been published relative to various studies on *Spirochaeta hyos,* an organism present in certain hog cholera lesions. The investigation, which is still incomplete, has been continued since the publication of the first preliminary paper in 1913. The present paper has been prepared for the purpose of reporting additional data and presenting a general summary of the investigation.

EXPERIMENTAL NOTES. *Presence of Spirochaeta hyos in blood of cholera infected hogs.* The study of spirochetes found in swine was prompted by the results of early observations on the blood of cholera infected hogs which were reported [1] [2] as follows:

"In the specimens of blood from all infected hogs, which have been observed by means of the dark field, a relatively large spirochete has been found. It averages from five to seven microns in length and one micron in width. The body of the organism is flexible and round at its ends. It presents no knobbed appearance at its poles. Actively motile, it revolves about its longitudinal axis. Its motility is undulating in character and its spirals are fixed. A few of these organisms have been observed dividing longitudinally. In one permanent microscopical mount, prepared by India ink fixation, one of these organisms apparently shows a polar flagellum. On the dark field this spirochete is readily distinguished from bacteria on account of its lack of rigidity and its characteristic motility, and from 'blood filaments' by its greater refractive properties and characteristic morphology.

"This spirochete has not been found in large numbers, in any of the blood preparations. However, in nearly every specimen examined, more than one have been observed, and in many cases five or six have been found with little difficulty. As a rule the organisms have been found to be more numerous at the height of the disease. The specimens of blood examined have been diluted in the proportion of about one to ten or fifteen with sterile sodium citrate solution, which factor should be considered in contemplating the number observed in a given positive specimen. Moreover, it is suggested that this organism, when

*Read before the meeting of the Am. Vet. Med. Assn., Detroit, 1916.

observed as a spirochete form, constitutes only one stage of its development."

Since reporting the blood findings resulting from the microscopical study on the dark field, no further data on this phase of the problem has been published. Subsequent work dealt with studies of spirochetes in the intestinal mucosa and local lesions, cultural studies, filtration and inoculation experiments and complement fixation tests. On numerous occasions, however, attempts were made to secure satisfactory stained preparations as well as cultures of the spirochete from the blood. These attempts were wholly unsuccessful. No growth of the spirochete occurred in cultures made from the blood in various special deep tube media, such as serum, blood, agar, ascitic and amniotic fluid in various combinations. A few months ago attention was directed to this particular phase of the problem because it seemed necessary not only to verify the results of the microscopical study of the blood, but also to investigate the possibility of securing pure cultures of the spirochete directly from the blood of cholera infected hogs.

Pure cultures of *Spirochaete hyos* may be obtained from the intestinal ulcers or local lesions of cholera infected hogs by making repeated transfers from the original culture. Owing to the period of time necessary to eliminate contaminating organisms from the original cultures, the final transfers containing the spirochetes in pure cultures are often unsatisfactory for animal inoculation experiments. This is an assumption based upon the probable attenuation of the cultures because of long continued passage on artificial culture media. Since the spirochete had been found in the blood by means of the dark field, it appeared that by proper methods the organisms might be isolated in pure culture directly from the blood and thus afford unattenuated cultures for animal inoculation and other experiments. Failure to grow the spirochetes from the blood in tube media led to the use of mass cultures.

Culture media. The culture media used for the earlier blood cultures consisted of a mixture of 100 c.c. of veal bouillon (5 points acid to phenolphthalein); 50 c.c. sterile ascitic fluid and 25 c.c. of 2% veal agar in a flask containing 4 or 5 small pieces of fresh, sterile rabbit testicle or kidney tissue. Considerable dif-

ficulty was encountered in putting up this media due to the nature of the ingredients, and the impossibility of sterilizing the mixture. Other formulae, using varying amounts of the same material, with and without sterilization, were tried later.

Owing to the difficulty in obtaining ascitic fluid in a sterile condition, attempts were made to replace it with amniotic fluid obtained from pregnant sows slaughtered at the abattoir. This was used both with and without sterilization and in approximately the same amounts as the ascitic fluid. Some promising results were obtained, but it was not found to be as satisfactory as the ascitic fluid. Fluid obtained aseptically and used without sterilization, gave much better results than that sterilized.

The formula determined upon for use in the later cultures was as follows:

Ascitic Fluid.....	40 c.c.
Amniotic Fluid.....	50 c.c.
Agar	25 c.c.
Bouillon 100	c.c.

These ingredients were mixed in a 250 c.c. flask to which the tissue had been added and the whole mixture was autoclaved.

Technic involved in making cultures. (1) Tail bleedings; the tail was washed carefully with mercuric iodide soap, shaved and treated with a mixture* of alcohol, acetone and coal tar product possessing high phenol coefficient. The end of the tail was then cut off and the blood caused to flow in a thin stream directly into the culture flask.

(2) Aspiration from the heart; the skin over the heart was washed, shaved and painted with iodine. Then by means of a sterile syringe fitted with a long needle, the blood was aspirated directly from the heart and forced into the flasks from the syringe.

(3) Slaughter bleeding; the animal was stunned by a blow on the head, the thorax quickly opened and the blood sample aspirated from the pulsating heart by means of a sterile 25 c.c. pipette.

In all cases, at least 25 c.c. of blood were taken, if possible, and after mixing with the media a layer of sterile neutral oil was added. When the blood was drawn, control cultures were made on nutrient agar and bouillon, to aid in determining its sterility.

*See article by McDonald, *Jour. Surgery, Gynecology and Obstetrics,* Vol. 21. July, 1915, p. 82.

Incubation and examination of cultures. The cultures were incubated at 37° for several weeks. Examination by means of the dark field were made at intervals of a day or two for some time and then at less frequent intervals. Whenever the spirochetes developed, they were found from four to twenty-nine days after the cultures were made.

The contaminating organisms consisted of *B. coli communis, B. cholerae suis, B. enteritidis, B. subtilis,* staphylococci, streptococci, and several unidentified bacilli and spirilla.

When the cultures showed growth of active spirochetes, transfers were made into ascitic agar tubes, containing tissue. In some instances, owing to the presence of very rapidly growing bacteria with gas production, the spirochetes did not develop and usually the purification was most difficult.

Strains of Virus Utilized. Four strains of virus have been used in these experiments:—

1—New York, received from Drs. Moore and Birch of Cornell University.

2—St. Louis, received from Dr. Houk of East St. Louis, representing stock strain built up by mixing together all the strains of virus obtainable.

3—Wisconsin, received from Drs. Hadley and Beach of the University of Wisconsin.

4—Grosse Isle, obtained from a herd of cholera infected hogs at Grosse Isle, Mich.

Table I.

GENERAL DATA, BLOOD CULTURES.

No. Flask.	Hog No.	Strain Virus	Character of bleeding	No. days after inoc. when cults. made	No. days before Spirochete observed
1	248	N. Y.	Heart (1)	15	11
2	254	St. L.	Tail	8	
3	256	Wis.	Tail	8	
4	251	St. L.	Heart (1)	16	11
5	254	St. L.	Heart (2)	18	9
6	261	N. Y.	Tail	10	
7	256	Wis.	Heart (2)	18	
8	259	Wis.	Tail	10	9
9	255	N. Y.	Tail	20	
10	258	G. I.	Tail	12	
11	259	Wis.	Heart (2)	13	29
12	257	G. I.	Heart (2)	22	6

No. Flask	Hog No.	Strain Virus	Character of bleeding	No. days after inoc. when cults. made	No. days before Spirochete observed
13	255	N. Y.	Heart (1)	24	
14	262	St. L.	Tail	4	
15	265	G. I.	Tail	4	
16	263	Wis.	Tail	7	
17	264	N. Y.	Tail	7	
18	261	N. Y.	Heart (2)	20	29
19	258	G. I.	Heart (2)	20	
20	252	N. Y.	Heart (2)	36	
21	265	G. I.	Heart (2)	9	
22	262	St. L.	Tail	10	
23	263	Wis.	Tail	10	8
24	263	Wis.	Tail	12	18
25	264	N. Y.	Heart (2)	12	6
26	267	St. L.	Tail	5	
27	263	Wis.	Heart (1)	15	
28	262	St. L.	Heart (1)	17	9
29	266	N. Y.	Tail	10	
30	267	St. L.	Tail	10	
31	268	G. I.	Tail	10	
32	269	Wis.	Tail	10	
33	266	N. Y.	Heart (1)	16	4
34	267	St. L.	Heart (1)	16	
35	270	St. L.	Tail	8	
36	271	N. Y.	Tail	8	
37	273	G. I.	Tail	8	
38	269	Wis.	Heart (1)	22	4
39	272	Wis.	Tail	10	
40	271	N. Y.	Heart (1)	14	
41	273	G. I.	Heart (3)	15	
42	270	St. L.	Heart (3)	16	
43	278	Normal	Tail		
44	279	Normal	Tail		
45	280	Normal	Tail		
46	277	Normal	Tail		
47	272	Wis.	Heart (1)	18	12
48	268	G. I.	Heart (3)	28	6
49	277	G. I.	Heart (1)	3 or 4	
50	279	St. L.	Heart (3)	5	
51	281	Normal	Tail		
52	280	N. Y.	Heart (3)	6	12
53	270	St. L.	Heart (3)	23	
54	273	G. I.	Heart (1)	24	
55	279	St. L.	Heart (3)	8	
56	287	Normal	Heart (3)		
57	286	Normal	Heart (3)		
58	278	Wis.	Heart (3)	7	
59	281	G. I.	Heart (3)	9	
60	275	N. Y.	Heart (3)	7	
61	287	Wis.	Heart (1)	8	
62	285	G. I.	Heart (3)	11	
63	286	St. L.	Heart (3)	11	
64	281	G. I.	Heart (1)	10	
65	285	G. I.	Heart (3)	15	
66	286	St. L.	Heart (2)	15	9

No. Flask	Hog No.	Strain Virus	Character of bleeding	No. days after inoc. when cults. made	No. days before Spirochete observed
67	287	Wis.	Heart (1)	18	*95
68	284	N. Y.	Heart (3)	12	7
69	278	Wis.	Heart (4)	20	5
70	294	St. L.	Heart (3)	6	†90
71	291	N. Y.	Heart (3)	7	
72	293	G. I.	Heart (3)	8	
73	292	Wis.	Heart (3)	9	
74	294	St. L.	Heart (3)	12	
75	293	G. I.	Heart (3)	12	
76	291	N. Y.	Heart (3)	14	6
77	293	G. I.	Heart (3)	16	6
78	294	St. L.	Heart (3)	16	6
79	291	N. Y.	Heart (4)	19	7
80	300	St. L.	Heart (3)	10	6
81	298	G. I.	Heart (3)	11	
82	301	N. Y.	Heart (3)	12	
83	299	Wis.	Heart (3)	12	
84	300	St. L.	Heart (4)	13	
85	299	Wis.	Heart (4)	16	5
86	298	G. I.	Heart (4)	16	15
87	304	St. L.	Heart (3)	9	7
88	303	G. I.	Heart (3)	10	
89	305	N. Y.	Heart (3)	12	
90	302	Wis.	Heart (3)	13	13
91	288	St. L.	Heart (3)	23	
92	304	St. L.	Heart (3)	16	10
93	290	Wis.	Heart (2)	18	
94	297	Wis.	Heart (2)	18	23
95	295	Wis.	Heart (3)	18	5
96	306	St. L.	Heart (2)	38	17
97	312	N. Y.	Heart (2)	10	8
98	313	St. L.	Heart (2)	10	18
99	311	G. I.	Heart (2)	12	12

(1) Blood taken from the heart with 25 c.c. pipette after death.
(2) Blood taken from the heart with 25 c.c. pipette while animal was stunned.
(3) Blood aspirated from the heart by syringe while animal was alive.
(4) Blood taken from the heart by syringe after death.

A summary of the results shown in TABLE I may be expressed as follows:

1. No spirochetes could be found in cultures made from the blood of normal hogs although contaminating bacteria were present in practically all cases.
2. No spirochetes were observed in blood cultures made from 1-5 days after inoculation of hogs with the filterable virus.

*Culture not examined after 19 days.
†Culture not examined after 22 days.

3. 15.8 per cent of total blood cultures made from 6-9 days after inoculation of pigs with the filterable virus resulted positively.
4. 50 per cent of total blood cultures made 10 or more days after inoculation gave positive findings.
5. 53 per cent of total blood cultures made after death from hog cholera contained *Sp. hyos.*
6. *Sp. hyos* were observed in all positive cultures from 4 to 29 days after cultures were made, with two exceptions.

It should be borne in mind that the foregoing series includes all mass blood cultures since the beginning of the work.

For the first several weeks considerable experimentation was carried out for the purpose of developing the most satisfactory culture media formula, correcting errors in technique and devising the best methods for the elimination of contamination, in so far as possible. Notwithstanding these difficulties 40 per cent of the total blood cultures showed the presence of *Sp. hyos.* Most of the cultures, especially those in the first part of the series, contained many contaminating organisms and it is quite probable that in some instances the spirochetes may not have developed because of overgrowth of extraneous forms.

It is practically impossible to secure relatively large blood specimens from the hog, uncontaminated, therefore undue importance cannot be attached to the foregoing results. However, the general results corroborate those previously reported from the dark field study of the blood of normal and cholera infected hogs.

Pathogenicity of Spirochaeta Hyos. References have been made in published data[3 4] to the successful production of symptoms and lesions similar to those of hog cholera by means of inoculation with contaminated and pure cultures of *Sp. hyos.* A summary of these reports is presented in Table II.

TABLE II.

SUMMARY OF PUBLISHED DATA, ANIMAL INOCULATION EXPERIMENTS.

Hog	Character of Cult. of Sp. Hyos: Pure or contam.	Character of Cult. of Sp. Hyos: No. of generation on cult. media	Age of transfers days	Results
559	contam.	1st	5	Disease typical of H. C. Check animal inoc. with contam. cults., remained normal. Berk. filtered serum 559 produced disease similar to H. C.
622	contam.	2nd	57	Mild reaction.
623	contam.	2nd	57	Mild reaction.
612	contam.	2nd	22	Disease typical of chronic cholera. Berk. filtered serum 612 produced disease similar to H. C.
613	contam.	2nd	22	Disease typical of H. C. Berk. filtered serum 613 produced same in normal pig.
614	contam.	2nd	22	Disease typical chronic chol. Berk. filtered serum 614 produced symptoms similar to those of mild chol.
627	contam.	3rd	36	Strong reaction, gained resistance against H. C.
628	contam.	3rd	36	Strong reaction—blood produced disease typical of H. C.
642	contam.	3rd	52	Disease typical of mild cholera.
643	contam.	3rd	52	Disease typical of mild cholera.
805	pure from filtrate	2nd	70	Disease typical of H. C. Controlled by check animal.
806	Pure from filtrate	2nd	70	Disease typical of H. C. Controlled by check animal.

During the past year 27 hogs have been subjected to inoculation experiments with *Sp. hyos*. The methods used in conducting these experiments and in controlling the results are illustrated by the following laboratory data:

EXPERIMENT II.—Hog 121 received injections of pure cultures of *Sp. Hyos* as follows:

Hog 121—5-27-15. Intramusc. Inj. 6 c.c. susp. trans. II 112
6-1 -15. Intramusc. Inj. 5 c.c. susp. trans. III 112
6-4 -15. Intramusc. Inj. 4½ c.c. susp. trans. III 112
6-8 -15. Intramusc. Inj. 8 c.c. susp. trans. III 112

Repeated inoculations of pure cultures were made for the purpose of comparing results with those from single inoculations. Flexner[5] and his associates found that several intraperitoneal and intraspinal inoculations of monkeys with cultures of the organism

which they have isolated from cases of acute anterior poliomyelitis, were required to produce infection.

This animal showed a thermal and constitutional reaction 6-2-15 to 6-6-15, after which it regained normal conditions. Subsequent exposure to the filterable virus proved that it was immune to hog cholera.

Hog 123 received injections of pure culture of *Sp. hyos* as follows:

Hog 123—5-27-15.	Intramusc. Inj.	4½	c.c. susp. trans.	II	112
6-1 -15.	Intramusc. Inj.	4	c.c. susp. trans.	III	112
6-4 -15.	Intramusc. Inj.	5	c.c. susp. trans.	III	112
6-8 -15.	Intramusc. Inj.	10	c.c. susp. trans.	III	112

This animal showed very slight reaction from the cultures injected 6-2—6-5, on 6-8 exhibited marked symptoms similar to those of the subacute type of hog cholera and died 6-23-15.

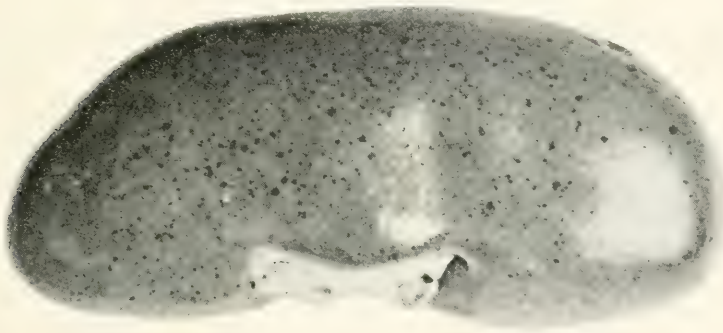

FIG. 1. Kidney.

Hogs 122 and 124 were placed with hogs 121 and 123 at the beginning of the experiment, 5-27-15.

Hog 122 on 6-10-15 developed symptoms typical of H. C. which proved fatal in 18 days. It is significant to notice that this animal showed symptoms just 6-7 days after hog 121 exhibited a strong reaction from injection of cultures.

Hog 124 remained normal until 6-16, 7-8 days after hogs 123 and 122 manifested symptoms, evidently not becoming exposed from 121 at the same time as hog 122. With the exception of 121 all suffered from disease processes typical of H. C.

Autopsy Hog 122.

Pericardial sac—ecchymotic spots.

Heart muscle—irregular hemorrhagic areas.

Lungs—small hemorrhagic areas.

Spleen—enlarged, soft and engorged.

Glands, mediastinal, inguinal and mesenteric—enlarged and hemorrhagic.

Kidneys—petechiae, "turkey egg." See Fig. 1.

Cecum—Mucosa ulcerated around ileo-cecal valve. Small hemorrhagic areas. See Fig. 2.

Autopsy Hog 123.

Lungs—hemorrhagic areas.

Spleen—very large and friable.

Glands—mesenteric and inguinal enlarged.

Kidney—few ecchymotic spots.

Cecum—two small ulcers.

Liver—normal.

Heart—normal.

Autopsy Hog 124.

Lesions correspond with those found in hog 122 with the addition of petechiae on the ental surface of the bladder. See Figs. 3, 4, 5.

Experiment 12.—Hog 131 received 12 injections of liquid cultures of *Sp. hyos* during a period from 6-15—7-25-15. The animal remained in normal condition and was found later to be immune to hog cholera.

Hog 132 placed in the same pen as a control throughout the experiment also remained normal and was found to be immune upon exposure to the filterable virus of H. C.

Hog 133—Another control, remained normal or nearly so for almost two months and then gradually developed symptoms characteristic of the chronic type of hog cholera to which it succumbed 9-8-15. Autopsy showed characteristic lesions.

Hog 134 received 12 injections of liquid cultures of *Sp. hyos* during a period from 6-15—7-28. As a rule this animal showed thermal reactions after each injection, manifested symptoms 8-3-15 and died 9-9-15. The autopsy showed lesions similar to those of H. C.

Hogs 137 and 138 were given 7 injections and fed cultures of *Sp. hyos* during a period from 6-15 – 7-12. After about 11 weeks both of these animals manifested symptoms of disease.

No. 137 recovered and proved immune to hog cholera.

No. 138 died of a chronic type of disease similar to H. C. 9-17-15. The experiments on these animals (137 and 138) were controlled throughout by the presence of normal hogs (135 and 136) which developed an immunity to the filt. virus of H. C., presumably through exposure to the attenuated organisms causing disease in 137 and 138. The accompanying photograph (see Fig. 6) shows the condition of the animals included in this experiment on 9-10-15.

Fig. 2. Cecum.

Hogs 133 and 137 do not appear in the photograph, as No. 133 died on 9-8 and was destroyed, while No. 137, in normal condition, was not in the field of the camera. Hog 134 had died the previous day, while hog 138 was very sick.

TABLE III.

SUMMARY OF UNPUBLISHED DATA.
ANIMAL INOCULATION EXPERIMENTS.

	Hog No.	Character of Cult. of Sp. Hyos. Pure or Contam.	No. of generation on cult. med.	Age of Transfers. (days)	Result of Experiment
1	53	Cont.	2	9	Acute disease, died 14th day.
2	56	"	2	18	Subacute disease, died 17th day.
3	58	"	5	5	Chronic disease, died, control, acute type.
4	78	Pure	16	8	Subacute, died 23rd day, control died.
	79	"	16	8	Chronic, died, control died.
5	82	"	16	8	Chronic, died, control died.
6	83	"	18, 19 & 20	4, 7, 20, 30	Not affected by cult., died after exposure to filt. virus.
	92	"	18, 19 & 20	4, 7, 20, 30	Not affected by cult., died after exposure to filt. virus.
7	95	"	4 & 5	6, 22, 25, 28	Not affected by cult., died after exposure to filt. virus.
	97	"	4 & 5	6, 22, 25, 28	Immunized by cult. to filt. virus.
8	103	"	7, 8 & 9	3, 20, 23	Immunized by cult. to filt. virus.
	104	"	7, 8 & 9	3, 20, 23	Died of subacute disease.
9	108	"	10 & 11	4, 7, 11	Not affected by cult., died after exposure to filt. virus.
	110	"	10 & 11	4, 7, 10	Not affected by cult., died after exposure to filt. virus.
10	115	"	6	22, 28	Chronic disease, died, controls, acute disease.
	116	"	6	22, 28	Chronic disease, died, controls, acute disease.
11	121	"	2 & 3	2, 6, 7, 11	Immunized by cult. to filt. virus, control died.
	123	"	2 & 3	2, 6, 7, 11	Chronic disease, died, control died.
12	131	"	3, 4, 5 & 12	21, 3, 6, 2, 6, 4	Immunized to filt. virus, control died.
	134	"	3, 4, 5 & 12	21, 3, 6, 2, 6, 4	Acute disease, died.
	137	"	3, 4, 5 & 6	11, 3, 8, 17, 6, 6	Reaction only from culture –immune to filt. virus.
	138	"	3, 4, 5 & 6	11, 3, 8, 17, 6, 6	Reaction only from culture. Chronic dis., died after exp. to filt. virus.
13	140	"	4, 5, 6, 9, 11	6, 22, 7, 11, 14, 10	Immunized by cult. to filt. virus.
	145	"	4, 5, 6, 9, 11	6, 22, 7, 10, 14, 10	Not affected by cult., acute H. C. after exp. to filt. virus.
14	275	"	5	3, 6	Died while being bled. Had reaction and symptoms.
	282	"	5	3, 6	Strong reaction, immune on expos., 2 controls immune.
15	306	Contam.	2	16	Reaction from cult. Control, after exposure during reaction period, took subacute type of disease, and died. Cult. pig developed acute type of disease and died after symptoms appeared in control.

In the foregoing series of inoculations of 27 hogs with cultures of *Sp. hyos,* each of which was controlled by one or more normal pigs, except in the case of numbers 53 and 56, the following summarized results were obtained:

12 hogs (45.2%) developed disease typical of hog cholera, the cases terminating fatally.

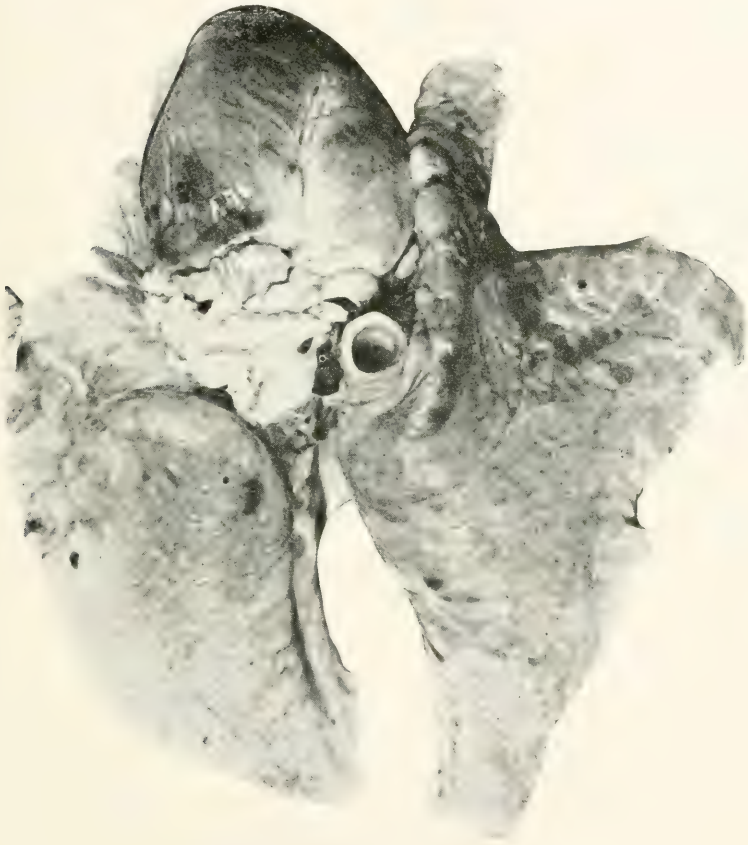

FIG. 3. Heart and Lungs.

7 hogs (26%) exhibited reactions, after which they were injected with the filterable virus and found to be immune to hog cholera. 7 hogs (26%) were not affected by the injection of cultures of *Sp. hyos,* as shown by absence of immunity on subsequent exposure to the filterable virus.

1 hog (3.7%), number 275, gave questionable results.

In these experiments nine different culture strains of *Sp. hyos* were used.

It is a well known fact that *Spirochaeta pallida* rapidly loses its virulence when cultivated on artificial culture media.

In the discussion of "Immunity in Syphilis," Zinsser[6] makes the following statement:

"Were it not for the production of lesions with cultures in their early test tube generations by Hoffman, and by Noguchi in a few experiments, one would be almost in doubt as to the identity of the virulent with the culture organisms."

Further study may show that *Sp. hyos* may exhibit the same characteristic.

The results of animal inoculation experiments indicate that *Spirochaeta hyos,* when injected into healthy hogs, in some instances is capable of producing symptoms and lesions similar to those present in hog cholera.

Further experimentation is necessary, however, in order that definite conclusions can be drawn as to the specific etiological relationship of this organism to hog cholera. At the present time the following tentative suggestions are offered:

1. *Sp. hyos* under certain conditions is pathogenic for swine, producing symptoms and lesions characteristic of hog cholera.

2. *Sp. hyos* may rapidly become attenuated when grown under artificial conditions and therefore may often become incapable of producing disease when injected into healthy hogs.

3. The injections of attenuated cultures into healthy hogs sometimes sensitize the animals and sometimes confer resistance against infection when subsequent inoculations are made with the filterable virus of hog cholera.

4. The results of several inoculation experiments have shown that control hogs confined with the inoculated animals may develop symptoms and lesions similar to those found in hog cholera, while inoculated hogs may remain normal or exhibit reactions only. This may be explained by assuming that the attenuated organisms multiply after injection, and, regaining their virulence in a natural habitat, may cause disease when ingested by the healthy control animals.

Summary of the Investigation of Spirochaeta Hyos. The general trend of results pertaining to this series of investigations

justifies a continuation of the study of *Sp. hyos.* Much work remains undone and some of the results which have been reported await the confirmation of other investigators. However, the findings bear some significance, and further investigation of this organism is warranted on the part of those interested in the problem of hog cholera, its cause and prevention.

Hog cholera is at present classified etiologically as a disease due to an ultravisible virus. There exists no recognized consensus of opinion regarding the relative importance of the patho-

Fig. 4. Mesenteric Glands.

logical changes which take place in cases described as hog cholera, and present literature on this subject constantly revolves around the query, "What is hog cholera?" The diagnosis rests entirely upon history of outbreak, clinical symptoms, autopsy findings and the results following treatment with serum. Preventive treatment depends upon the use of anti-hog cholera serum, which during the last six years has been manufactured in large quantities and used extensively. The value of anti-hog cholera serum has been abundantly proven, but the general, practical efficacy of the prod-

uct, in proportion to its cost and certain other disadvantages, has been subjected to serious criticism.

Students of the hog cholera problem must place more concentration upon the study of the filterable virus and the pathology of the disease in order that a logical basis may be established for the successful solution of the problems of accurate diagnosis and practical prevention.

General conclusions formed from results obtained thus far. During this series of investigations the following phenomena have been demonstrated:

1. The presence in small numbers, of a spirochete, described as *Sp. hyos,* in the blood of hogs infected with hog cholera, as shown by microscopical studies. In the majority of cases, mass cultures from the blood of animals suffering from hog cholera yield a growth of spirochetes, usually accompanied by *B. coli communis, B. cholerae suis, B. enteritidis,* staphylococci and other organisms.

2. The presence of *Spirochaeta hyos* in the intestinal ulcers, cecal crypts and external local lesions of animals suffering from hog cholera.

3. The successful filtration of *Sp. hyos* from suspensions containing a variety of contaminating microörganisms, as evidenced in one or two instances by growth of the spirochete in pure culture in transfers made from the filtrates, and the production of disease processes similar to hog cholera in hogs inoculated with the filtrates.

4. The production of disease similar to hog cholera, under certain conditions, in normal hogs inoculated with pure cultures of *Sp. hyos* which have passed through several generations on artificial culture media. The presence of sensitization in some instances and, in others, resistance toward subsequent injections of the filterable virus of hog cholera, in hogs which have received pure attenuated cultures of *Sp. hyos.*

5. The specific antigenic power[7] of extracts prepared from pure cultures of *Sp. hyos* when used in complement fixation tests with sera from cholera hogs as compared with the results of similar complement fixation tests with the sera of normal hogs and those suffering from other disease processes.

Confirmatory Data. Relatively little work has been reported in confirmation of the foregoing conclusions.

Arnheim[8] found spirochetes on dark field preparations made from the peripheral blood of infected hogs, but not from the heart's blood. He could not demonstrate spirochetes in the blood of normal hogs.

Connaway and Durant[9] have found that an experimental antigen prepared from intestinal ulcer material, which is usually rich in spirochetes, gave encouraging results as a specific reagent in experimental complement fixation tests.

This experimental ulcer extract antigen was prepared in such a way that it was "practically free from blood and blood carrying tissue containing any large amount of the circulating "filterable virus."

Connaway and Durant also tested a total of 76 experimental spleen extract antigens, 10 per cent of which manifested anti-

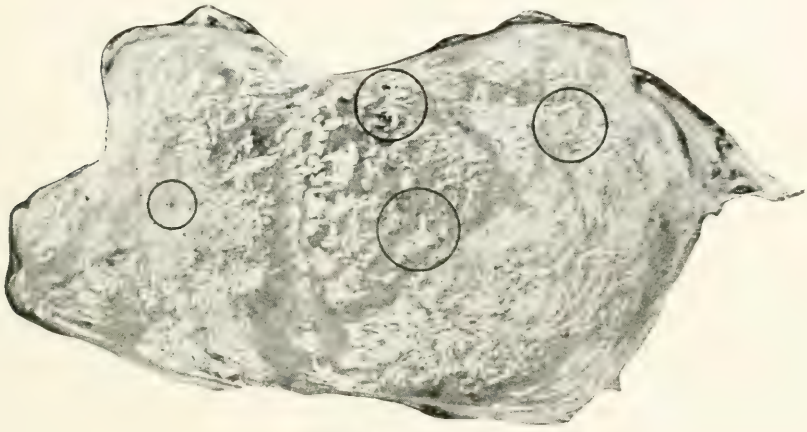

Fig. 5. Bladder.

genic properties. Experimental antigens prepared from the blood, kidneys and lymph glands yielded negative results.

Rüther[10], Betegh[11], Uhlenhuth and Haendel[12], Arnheim[8] and Dorset[13] report the presence of spirochetes in the intestinal ulcers of infected hogs. Uhlenhuth and Haendel state that spirochetes have been found in the bile of infected hogs.

In former publications[4] and [14], reference has been made to a short article published by Rüther[10] concerning the presence of spirochetes in the intestinal mucosa of cholera infected hogs. In a monograph, "Zur Sichtbarkeit des Schweinepest-erregers,"

printed by Rüther in 1910, he reported the observation of spirilla in the blood of cholera infected hogs, referred to the passage of these organisms through a filter and suggested the etiological significance of the spirilla in hog cholera. In this monograph the term "spirochaeta" was suggested in one instance as synonymous with the term "spirilla," but no attempt was made to describe a definite microörganism.

Criticisms. Some criticisms have been offered concerning the findings summarized in the foregoing. Such have been expressed, for the greater part, merely as opinions and not as facts based upon actual data.

While Arnheim[8] verified the statement that spirochetes may be demonstrated in the blood and intestinal mucosa of cholera infected animals, he expressed the belief that *Sp. hyos* is a saprophyte. He based this conclusion upon the fact that treatment of hog cholera with salvarsan results negatively and also upon the assertion that spirochetes had not been passed through filters under pressure, at that writing.

Fig. 6. Sick Hogs.

Hayes[15] states that spirochetes could not be demonstrated on the dark field in 12 specimens of blood from cholera infected hogs.

Dorset[16] reports that he was not able to find spirochetes in the blood of infected hogs. He states that spirochetes can be demonstrated readily in the intestinal mucosa not only of infected hogs but also that "an extended examination of the intestinal contents of healthy hogs has shown that the same spirochetes are present there in considerable numbers.

He therefore believes that *Spirochaeta hyos* is a "saprophytic inhabitant of the hog's intestine."

Meyer[17] calls attention to the destructive action of sodium taurocholate, saponin and lecithin on spirochetes, while the filterable hog cholera virus exhibits more resistance to such substances. This fact, according to Meyer, supports the opinion that *Sp. hyos* is not a pathogenic organism.

Referring to the negative evidence which has been advanced the following suggestions may be offered:

1. Failure to find *Sp. hyos* on the dark field in the blood of cholera infected hogs is an admission of lack of perseverance in following up the work. The spirochete may be secured in blood cultures as reported in this paper.

2. Failure to successfully treat cases of hog cholera with arsenical or mercurial preparations should not be considered as evidence that *Sp. hyos* is in no way related to hog cholera. The successful use of salvarsan in the treatment of syphilis is not uniformly accepted, and moreover syphilis is a disease which shows no clinical resemblance to hog cholera, and the successful treatment with mercury and salvarsan depends upon continued application of these substances.

3. Claims of failure to pass spirochetes through filters under pressure cannot be maintained at the present time.

Sp. hyos has been passed through the Berkfeld filter[18]. Wolbach and Binger[19] have described a spirochete, named by them *Sp. elusa,* which they passed through a Berkfeld "V" filter. They have also reported the filterability of *Sp. biflexa,* which they found in fresh water.

Todd and Wolbach[20] have shown that *Sp. duttoni* could be forced through a Berkfeld filter with a pressure of 50-90 pounds per sq. inch, while they did not pass through the filter under atmospheric pressure. It is inferred that the organisms which passed through the filter under pressure represented the "granule phase."

4. The demonstration of spirochetes in the intestines of normal hogs, with the assumption that such spirochetes are identical with *Sp. hyos,* does not constitute sufficient evidence upon which to draw negative conclusions regarding this organism.

It is now recognized that *Sp. pallidum* is the specific cause of syphilis in spite of the fact that the oral cavity of the healthy

individual is the normal habitat of saprophytic spirochetes. Positive findings of spirochetes in the intestinal mucosa of swine immune to cholera have been reported, but they are found in small numbers and only in rare cases in the intestines of healthy hogs, as compared with the findings in the intestinal ulcers and cecal crypts of infected animals. However, granting that spirochetes may be demonstrated in large numbers in the intestinal canal of healthy hogs, the assumption is not well founded that all microbic forms of the intestinal flora which resemble spirochetes morphologically, are identical with *Sp. hyos.* With our present meager knowledge of spirochetes, it is impossible to rely upon microscopical characters alone in distinguishing the known forms. As an illustration it may be stated that the intestinal tract of normal man contains non-pathogenic organisms so closely resembling *B. typhosus* microscopically that no differentiation can be made, consequently typhoid fever cannot be diagnosed in the laboratory by a morphological study of the organism.

During the course of this investigation, *Sp. hyos* has been found in large numbers in the intestinal mucosa of a few animals which had been immunized to hog cholera. It can be assumed that the presence of these organisms in the intestine of immune hogs may possibly characterize such animals as "carriers" of the disease.

5. The inference drawn from the data concerning the resistance of the filterable hog cholera virus to the action of sodium taurocholate as compared with that of spirochetes, like some of the foregoing, represents circumstantial rather than direct evidence. Much has been written concerning some of the phenomena attending the life history of *Sp. pallidum* and other spirochetes. While little is known concerning the life cycle of this group of organisms there are many well known authorities, Balfour[21], Fantham[22], Marchoux and Couvey[23], Hindle[24], Dutton and Todd[25], Sergent and Foley[26], Noguchi[27], who have observed the "granule phase." It is altogether possible that at certain stages of development *Sp. hyos* and other organisms of this group may be more resistant to various substances and conditions than during the period represented by the morphological form of the organism with which the worker is familiar.

General Conclusion. While much work remains to be done on *Spirochaeta hyos,* only one important step is necessary to prove

with certainty whether or not this organism bears any direct etiological relation to hog cholera. Some means must be developed through which the organism may be isolated in pure culture directly from the infected animal in order that pure cultures, unattenuated by tedious manipulation in an artificial environment, may be utilized in a series of animal inoculation experiments. Under such conditions, uniformly positive results, together with data already in hand, would serve as final, conclusive evidence as to the specific pathogenicity of *Sp. hyos.* Until such experiments can be successfully carried out, positive conclusions must be withheld, but in the meantime *Sp. hyos* may be regarded as an organism present in animals infected with hog cholera, possessing certain characters suggestive of its pathogenic nature.

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TRICHLOR-TERTIARYBUTYL ALCOHOL ANESTHESIA.*

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It is now nearly thirty-five years since Willgerodt (1) discovered (1881) that the action of caustic alkalies on a mixture of chloroform and acetone causes the formation of a crystalline compound somewhat resembling camphor in odor and taste. This compound (trichlor-tertiarybutyl alcohol, $C_4H_7OCl_3$) was first used extensively in this country by Abel in 1892. In 1895 he and T. B. Aldrich gave a report of their investigations into the pharmacological and chemical behavior of the drug in the animal organism, with practical demonstrations, before the American Physiological Society. These authors did not, however, publish their results, and the only existing evidence of their interest in the drug is the title of their presented paper as recorded in the reported proceedings (2) of the annual meeting of the American Physiological Society in 1895. From this time on the drug was used here and there throughout this country as an anesthetic for laboratory use. Meanwhile, in Europe, Kóssa (3) also had investigated the compound pharmacologically and discovered its properties as a local anesthetic. Zoltán von Vámossy (4, 5) carried the pharmacological work still further and also demonstrated the safety of this anesthetic by clinical experiments.

It was not until 1899, however, that the preparation of this compound was made practical, and at that time it was placed upon the market under the name "Chloretone." Just previous to this the chemical and pharmacological properties of the drug were again studied by Houghton and Aldrich (5, 6), and its marked hypnotic, anesthetic and antiseptic properties were verified. The anesthetic properties of the drug both general and local appeared to be of the greatest importance and consequently received the most attention. Much has been learned by practical experience with the use of trichlor-tertiarybutyl alcohol in the years following its discovery, and it is largely with its hypnotic and general anesthetic action that this article will deal.

*The trade name of this drug is "Chloretone."

The early pharmacological investigation of trichlor-tertiarybutyl alcohol showed that, whether administered per stomach, subcutaneously, intramuscularly, intraperitoneally, intravenously, per rectum, or by inhalation of the sublimed vapor, all degrees of hypnosis to complete general anesthesia were produced in laboratory animals, depending upon the amount of the substance given.

The use of trichlor-tertiarybutyl alcohol as a general anesthetic in human surgery was not advocated because of the very prolonged action of the drug and the consequent slow return to consciousness, since no antidote for its action has yet been discovered. Also the large dose necessary to produce general anesthesia with this drug alone, involves the risk of its toxic effect finally becoming evident. However, for several reasons which will be enumerated later, the use of trichlor-tertiarybutyl alcohol as an anesthetic in experimental pharmacology or physiology where the recovery of the animal is not absolutely essential has proven almost ideal. In cases where the minimum lethal dose of the drug has been slightly exceeded, the final toxic action is so delayed as to permit of the use of the animal for several hours. In such cases the respiration gradually becomes slower and slower and the animal dies from respiratory paralysis twelve to twenty-four hours after the administration of the drug. The pulse rate is only slightly lessened and the heart action remains good until the animal begins to suffer from lack of oxygen. Consequently the blood pressure is neither depressed nor stimulated, and the anesthetic is particularly suited for experiments upon the blood pressure. The very steady plane of anesthesia, which is maintained for a number of hours by one injection of trichlor-tertiarybutyl alcohol, is a very important advantage in its use in animal experiments. This is in direct contrast to the somewhat fluctuating anesthesia and consequent variable blood pressure that is obtained with the volatile anesthetics, ether and chloroform, except under the most expert methods of administration.

The satisfactory use of trichlor-tertiarybutyl alcohol as an anesthetic is attended with some minor difficulties largely because of its physical properties. Constant use of and experimenting with the drug for more than fifteen years have succeeded in overcoming these difficulties to a large degree. In the first place the slight solubility of trichlor-tertiarybutyl alcohol in water, it being soluble to the extent of about 0.6 of 1 per cent at 20° C.,

and the large dose required for general anesthesia greatly influence the administration of the drug. Anesthesia can be obtained by the use of a saturated aqueous solution given per stomach, but the large dose necessitates such an amount of the solution that absorption is slow and uncertain, particularly if the animal's stomach is filled or partially filled with food at the time of the dosing. This method was thoroughly tried out and discarded in favor of one in which hypodermic administration was used.

This drug is soluble in oils such as olive oil, white neutral oil, oleic acid, etc., and the administration of such a solution by hypodermic injection into the peritoneal cavity was used for some time. By warming the oil in a small dish, the proper weight of the drug can be easily dissolved and quickly administered. It was found, however, that the absorption from the oil solution was sometimes slow and uncertain, requiring several hours to produce general anesthesia. This objection was very largely overcome by the use of alcohol as a solvent. Trichlor-tertiarybutyl alcohol is very soluble in alcohol, but the irritating action of alcohol upon the tissues is a very important factor. It is possible, however, to prepare a 40 per cent solution of the drug in partial alcoholic solution and the local irritation caused by its hypodermic injection is not serious enough to warrant consideration in animal experimentation (where the animal is not allowed to recover).

The following procedure has been found very satisfactory for anesthetizing experimental animals, particularly dogs, which are to be used for blood pressure experiments. The dog is weighed and the dose, 0.4 gram per kilogram body weight (or about 3 grains per pound) is quickly measured and administered. The use of a 40 per cent solution of the drug is well suited to the purpose since 1 mil (1 Cc.) contains 0.4 gram and should, therefore, anesthetize 1 kilo weight of the dog. The 40 per cent solution is best prepared by dissolving the weighed amount of the trichlor-tertiarybutyl alcohol in as small an amount of alcohol as possible and afterwards bringing the solution up to the required volume by adding water and just enough alcohol to keep the drug in solution. The finished solution will usually contain 40 per cent to 45 per cent of alcohol by volume. Where much animal experimentation is done, it is best to prepare 100 to 200 mils of this 40 per cent solution since it is very stable.

A smaller dose of 0.3 gm. per kilo will often suffice to produce

the necessary anesthesia, but where rapid and complete action is desired and where the recovery of the animal after twelve to twenty-four hours is not essential to the experiment, I have found that the best results are obtained when the larger dose of 0.4 gram per kilo is given.

The administration of the dose is accomplished by chaining the dog to some stationary object and drawing the animal back by one hind leg, after which the injection of the drug into the peritoneal cavity can be quickly made without danger of being bitten if the dog is so inclined. Considerable delay and difficulty is encountered if the solution is injected into the bladder by mistake, but if the site of injection is well forward toward the diaphragm, the solution will never enter the bladder. The injection occasionally is followed by some evidence of the irritating action of the alcohol, but this is quickly overcome by the local anesthetic action of the drug. Within five minutes after the injection, the first results are noticed in a restlessness of the animal and some muscular incoördination. Very soon thereafter the animal is completely prostrated without a struggle except in very rare instances, and about twenty minutes after the injection complete anesthesia is obtained with the disappearance of the last reflex. Such a condition makes unnecessary the paralyzing of the vagi and the use of artificial respiration except, of course, in experiments involving the opening of the thorax.

The large dose of trichlor-tertiarybutyl alcohol (0.4 gram per kilo) acts quickly at first and unless it greatly exceeds the minimum lethal dose, death will not result for from one to three days. In animals of abnormally low resistance, which condition can usually be recognized by an emaciated appearance, it is best to give a considerably smaller dose and follow it one-half hour later with more of the anesthetic if necessary. Small dogs weighing less than 10 kilos require only about three-fourths as much of the drug as the healthy average sized specimen. Animals that are pregnant and in some instances those that have been pregnant usually require a much larger dose to bring about the required anesthesia, and are usually more or less unsatisfactory as experimental animals.

Trichlor-tertiarybutyl alcohol can be used in experiments where the recovery of the animal is desired, if a preliminary narcosis is produced by a hypodermic injection of morphine and

followed with an intraperitoneal injection of 0.2 gram trichlor-tertiarybutyl alcohol per kilo. This anesthesia will last four or five hours and recovery will be gradual but sure. Under these conditions more time is required to bring about the anesthesia and the nausea produced by the morphine is a disagreeable feature. However, there is the marked advantage that the operator can work without the aid of an anesthetist and that there is a steady plane of anesthesia for the work.

In summarizing the advantages of trichlor-tertiarybutyl alcohol as an anesthetic in animal experimentation, it can be briefly said that a dose of 0.4 gram per kilogram body weight injected intraperitoneally produces rapid and complete anesthesia lasting from twelve to forty-eight hours with the one injection. It is easily administered, requires no attention after the first dose, and gives a very steady plane of anesthesia which is well suited to blood pressure investigations or experimental surgery of all kinds. If the recovery of the animal is desired, morphine narcosis should be first produced and followed with one-half the above mentioned standard dose of the drug.

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THE PHENOMENON OF ANAPHYLAXIS: ITS CLINICAL SIGNIFICANCE AND PRACTICAL UTILIZATION.

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Consequent to a few distressing and unfortunate reports which have found their way into the lay as well as the medical press, and as a direct result of some erroneous interpretations of the early fatal experimentations on laboratory animals, an unfavorable impression of the meaning of the term anaphylaxis has been entertained by the public and is still fostered by a large majority of physicians. Therefore, it should be taught generally that anaphylaxis is not a subject of mere experimental research and theoretical considerations, to be thought of only with apprehension and misgivings, but one of most practical value and clinical importance.

The prevalent or popular conception of proteid sensitization is based on the classic picture of experimental anaphylaxis bequeathed us by the guinea-pig; a phenomenon rarely seen in the human subject and seldom observed in any animal other than the guinea-pig. It is a picture that belongs to the research laboratory and not to the bedside. A few cases of sudden death, following the therapeutic use of horse serum, have been reported, and the relation of some of these to anaphylaxis cannot be gainsaid, although it is extremely doubtful if more than a small percentage were a direct result of a sensitization to the proteid in question. Other abnormal conditions or pathological lesions have been found at autopsy to explain a large number of the fatalities; especially the condition of lymphatism, better known as *status lymphaticus.*

The fear of fatal results from the use of therapeutic sera that pervades the ranks of the laity should, by means of intelligent and constant education, be allayed, as ignorance on this subject no doubt contributes to the death of many individuals who otherwise might be saved. There is practically nothing to be feared from the serum itself, or the mode of its introduction into the system, provided proper precautions are observed by the physician. There is no question but that more die from antitoxin starvation in one month than will ever die of antitoxin anaphylaxis. According to statistics by Park, which have frequently

been quoted, the mortality from diphtheria in New York dropped from 36.4 per cent in 1893 to 10 per cent in 1905, a fall of 26.4 per cent. Also, there were only two sudden deaths out of over 50,000 cases following the injections of antitoxin. This means that if we allowed ourselves to be frightened by two fatal cases, over 26 per cent of all cases of diphtheria would die for the want of proper treatment. There are many other therapeutic agents that directly produce a much higher percentage of fatalities without their usefulness being seriously handicapped.

Experimental and clinical anaphylaxis are two entirely distinct conditions, due to the fact that proteid sensitization does not express itself in the same way in all animals. The manifesations in the guinea-pig are the most violent of the experimental animals, while the symptoms as recognized in man are relatively mild. According to Hektoen, the guinea-pig "gives the most constant and most intense symptoms, being four hundred times as sensitive as the rabbit." In dogs there is a rapid fall in blood-pressure, but not the respiratory symptoms as seen in the guinea-pig. In cattle there is an enormous edema of the udders and a cyanosis of the mucous membranes. It is said that the white mouse is practically refractory to proteid sensitization. Therefore, when a condition is known to differ so widely in various animals it is folly to attempt to compare one with the other and to judge the results of the injection of a proteid into one by the symptoms in another. When considering anaphylaxis, the human subject should not be made comparable to any other animal organism.

Nor must it be forgotten that the term anaphylaxis, as given to us by Richet, is a misnomer. Proteid sensitization or "allergy," as it is called by von Pirquet, is no longer regarded opposed to or against protection. All experiments seem to point to the fact that it is, after all, a form of prophylaxis and not anaphylaxis. The term anaphylaxis should no longer be used to designate the condition known as proteid sensitization. According to Bordet, proteid sensitization or, as it is unfortunately incorrectly termed, anaphylaxis "testifies to the fact that the struggle against a foreign element is taking place." Vaughan says: "Sensitization and immunity are different manifestations of the same process." Emery sums up the subject as follows: "It seems to me that we may regard anaphylaxis as a necessary step to the development of immunity to toxins—disadvantageous in itself, but of

advantage in that it represents a stage in the production of a more resistant condition." And Grinnan goes so far as to state that "prophylaxis depends upon anaphylaxis." It may or may not be a fact that prophylaxis depends upon anaphylaxis, that sensitization and immunity are different manifestations of the same process, but it is certainly true that it is the result of the attempt of the body to protect itself, and instead of being antagonistic to prophylaxis, is an expression of resistance, an indication of defense, and, therefore, a form of immunity.

Much has been written, from a theoretical standpoint, and a large amount of work is being done at the present time, relative to anaphylaxis, and while very little advance has been made as to the exact relationship of the proteid molecule to the phenomena, the fact is paramount that certain individuals are in some way hypersensitive to certain proteids. Personal idiosyncrasy and individual susceptibility are terms that have been used for years to denote certain conditions in individuals due to a hypersensitiveness to certain substances, either proteid or non-proteid. While the underlying causes of the various conditions included under these terms may be found ultimately to be identical, this idea cannot be reconciled with recent experimental data and clinical evidence, and, therefore, at present, proteid sensitization is considered to be a condition peculiar to itself.

In order to attempt to control the phenomena of anaphylaxis (including all proteid idiosyncrasies) for clinical and therapeutic purposes, experimenters have studied the problem from a practical standpoint, and many facts have already been learned relative to the prophylaxis and possible alleviation of some of the obscure conditions relative to proteid sensitization, and methods have been perfected for employing these same unknown phenomena for diagnostic purposes by adapting the many experimental and clinical findings to a practical end.

It has been known for a long time that when certain proteid substances are injected into certain individuals more or less of a reaction will be produced in that individual. It is said that that individual is hypersensitive to or has an idiosyncrasy for that proteid. That individual is, for some reason or other, unusually or abnormally sensitive to that proteid, and this shows or manifests itself by means of a reaction, either local or general, depending upon the quantity of proteid as well as the method and

site of injection. If the proteid is deposited upon mucous surface of the conjunctiva, administered cutaneously or intracutaneously, or massaged into the skin, the anaphylaxis will manifest itself as a local reaction at the site of application. If an injection is made subcutaneously the reaction may be local or general, depending upon the amount of proteid; and if given intravascularly, intradurally or intraperitoneally, the reaction will be general. Being specific, some of these reactions are employed daily, for diagnostic purposes, in a large number of known as well as obscure conditions.

It is an interesting fact that the first known observation of an anaphylactic or allergic phenomenon was that of a skin reaction made by Jenner in 1798. It was noted, during his experimental work with smallpox vaccine, that the animal organism, once having passed through an attack of either cowpox or smallpox, may respond to the cutaneous application of the virus, if made years afterward, by an "early local cuticular inflammation." This reaction was used many years later by von Pirquet as a basis for the construction of his theory of clinical allergy, and is now an every-day observation.

Possibly, also, one of the most important observations, from a clinical standpoint, was that of Koch, in the early part of his work, to the effect that an injection of tubercle bacilli under the skin of a tuberculous guinea-pig, instead of causing a progressive infection, would result in a necrosis and sloughing at the point of inoculation. This finally led to modifications which are now universally recognized as the tuberculin reactions.

From this time on stray observations relative to proteid sensitization were made, but it was not until the investigations of Richet (1902), Arthus (1903), and Theobald Smith (1904) were published that research along this line received its proper stimulus, resulting in the work of Otto, Rosenau and Anderson, Gay and Southard, Vaughan and Wheeler, and Nicolle. These investigators, all pioneers in the subject, were followed by a host of others, engaged in the many phases of the question, who have added a few new facts; up to the present time, however, the many data, gained clinically and experimentally, have not been pieced together into a theory offering anything like a satisfactory explanation for all the known phenomena of proteid sensitization or anaphylaxis. A number of theories have, nevertheless, been

advanced which may aid in the future elucidation of the problem, all of which are founded or based upon a single hypothesis, namely, in order for an individual to be subject to an anaphylactic condition, there must have been a previous parenteral injection of the homologous alien proteid.

The specific or diagnostic reactions, assumed as of a probable anaphylactic nature, which are related to diseases of micro-organismal origin, are all explained essentially according to the same principle, namely, that the primary infection sensitizes the individual—acting as the first or sensitizing injection—while the symptoms of anaphylaxis, following the application of the homologous proteid, whether ophthalmic, subcutaneous, intracutaneous, or cutaneous, are indicative of the reaction.

So long as the microörganismal proteids are present, it matters very little as to the exact method of preparation of the diagnostic material, as has been established by the fact that while various methods are being used, all are giving practically the same results.

Of the various tests of practical value that depend upon the anaphylactic reactions for their usefulness, the tuberculin tests are perhaps the most widely applied at the present time. The tuberculins employed for diagnostic purposes are essentially concentrated bouillon filtrates of a culture of the tubercle bacillus, or the desiccated proteids from the filtrate produced by precipitation and purification.

Another reaction, somewhat analogous to or resembling the tuberculin reaction, which is devoted to diagnosis of glanders in animals is the mallein reaction. The animal suffering with the disease is sensitized to the proteids of the specific microörganism, while the reaction, a local anaphylactic manifestation, is produced by the application of the mallein. Mallein, a bouillon filtrate prepared in a similar manner to the tuberculin, contains the proteids of the Bact. mallei.

The luetin test, proposed by Noguchi for the diagnosis of syphilis, is another reaction of like nature and of great diagnostic value. The proteid material in this case is represented by the entire culture media containing the devitalized spirochæta.

Other tests, depending upon microörganismal proteids, of less diagnostic importance at present, are the typhoid ophthalmic test of Chantemesse, Austrian, and others, and the typhoid cutaneous test of Link and others—the proteid material being either the

whole organism in bouillon, extracts of the organisms, or preparations made from the organisms. Other tests the value of which are not as yet fully established are the test of Iron for gonococcus infections, the diphtherin test as described by Kolmer, and possibly others.

The reaction after the use of bacterial vaccines for therapeutic purposes, termed the "negative phase," may likewise be of an anaphylactic nature; the infection sensitizing the individual while the injection of the suspension of dead bacteria produces the reaction—a general reaction in this case. It is a recognized fact that the larger the dose of vaccine the more severe will be the reaction. This reaction offers, in some instances, a means of diagnosis or of determining the specificity of the vaccine in question, and may be employed as well as an indication of the size of dose to be subsequently administered.

Other diagnostic reactions of an anaphylactic nature are related to vegetable and animal proteids. The question of a means of diagnosis for these various idiosyncrasies is one of the most interesting phases of the entire subject, and opens a field of exploration hitherto restricted to a few of the food proteids and the proteids of some of the common plant pollen.

The first of these susceptibilities to attract the attention of investigators was the condition known as hay-fever. This symptom-complex was found to be associated with the pollen of various plants, and later attributed to the proteids of the pollen as a result of investigations relative to the ocular and cutaneous tests. Sensitization may now be determined by means of any one of the tests previously cited, and resistance against this sensitization may be obtained by means of prophylactic subcutaneous injections of extracts of the pollen. Whether it results in the production of immunity, according to the universal interpretation of that term, or in an increase in the tolerance against the proteid, similar to an increased tolerance to any drug, such as arsenic, morphine, and others, is a question yet to be solved.

The fact remains, however, that a large per cent of individuals are either entirely or partially relieved of symptoms by means of prophylactic injections of extracts of pollen, although the treatment must be repeated seasonally. Individuals should be tested previous to treatment by either the cutaneous, intracutaneous, or ocular methods to determine to which proteid they are sensitive,

for otherwise they might be made hypersensitive to a proteid to which, normally, they are not sensitive.

Contrary to some authorities the author has found that aqueous extracts of the pollen do not deteriorate as rapidly as has been stated. If properly preserved they will retain their initial activity for at least a year.

The last two or three years a large amount of work has been done in an attempt to associate several obscure conditions, especially various skin lesions, with anaphylaxis. The most encouraging results have been obtained with eczema and the erythematous and urticarial rashes.

Strickler and Goldberg, White, Blackfan and others have found that sensitizations to certain foods are responsible for a large number of cases of eczema. This, of course, is an important finding, as it clears up a question that has long been puzzling the medical profession. In order to determine the article of food to which these patients are susceptible either the cutaneous or intracutaneous tests are resorted to.

According to White, only about 20 per cent of eczematous individuals do not appear sensitized to any of the common food types.

Blackfan found "of twenty-seven patients with eczema, twenty-two gave evidences of susceptibility to proteins," and that "the removal of some or all of the animal proteins from the food brings about great improvement in some cases of eczema in older children and adults."

McBride and Schorer found that "certain of the proteid foods, on ingestion by certain individuals, produce urticaria and erythemata and other symptoms," and that "each food produces fairly constant skin lesions . . . fish, tomatoes and cheese only producing urticaria, while cereals and pork produce erythema in a considerable proportion of the cases." "Treatment should be specific. After getting rid of parasites and disease, if there still is hypersensibility, then immunization by feeding is usually but not always possible."

Other skin lesions have been mentioned as probably due to food or other idiosyncrasies, but not enough work has been done on them to warrant any discussion at present.

A very important field has been opened up as regards the anaphylactic nature of many obscure asthmatic conditions or even

certain conditions simulating the hay-fevers. It has been found that many of these cases are sensitized to one or more proteids, and recoveries have been reported as a result of a total abstinence from that proteid, following a specific skin test.

Certain forms of emphysema and edema of the lungs are conditions of an obscure nature that may be proved to be of anaphylactic origin in the near future, although nothing definite, as yet, can be said to sustain this opinion.

As food for thought it might be mentioned that the view has been advanced that puerperal eclampsia, the crisis of pneumonia, and the acute exanthemata are in some way associated with the phenomenon of anaphylaxis. Most of the statements as regards these conditions are made merely as suggestions and are not fortified by any experimental data. It will be noted, however, that the period of incubation which characterizes all experimental phenomena of anaphylaxis is, in pneumonia, represented in the period from the time of infection into the crisis; also that the acute exanthemata have incubation periods and lesions of the skin that singularly typify those of proteid sensitization.

The question naturally arises as to the methods pursued for the protection against and relief of the various anaphylactic conditions found in the human subject. For the prevention of symptoms resulting from the injection of horse serum there is no doubt, if care is taken to test the sensitization of each individual to horse serum, by means of either the cutaneous or intracutaneous reaction, that a large number of patients might be saved the necessity of undergoing many of the disagreeable symptoms, and perhaps the number of fatal cases would be materially decreased. If the reaction is positive the patient is considered sensitive to horse serum, and extreme caution must be practiced in the administration of the serum.

Several methods have been suggested for the injection of serum in a patient known or suspected to be sensitive to horse serum, some of which are more practical than others. Besredka has advised the initial subcutaneous injection of a very small dose, about 0.1 Cc. to 0.2 Cc., followed within two or three hours by the full dose, providing no untoward symptoms have appeared. The anaphylactic shock is said to be prevented or suppressed by rectal injections of the serum or by means of subcutaneous injections given a few hours apart, starting with small doses and grad-

ually increasing them. It has also been advised to dilute the serum with about 500 Cc. sterile physiologic salt solution, giving the mixture very slowly by means of hypodermoclysis. It is considered much safer to have the proteid absorbed slowly, not allowing the entire amount to enter the body parenterally at once.

Several methods have been proposed for the prevention and cure of the various food idiosyncrasies, some of which have proved more satisfactory than others. The method of withdrawing from the diet the proteid in question or all proteids is practiced by some; this, however, has one great disadvantage in that it is not permanent. Another method advocated has for its object the gradual raising of the tolerance of the individual by feeding the proteid in question or by rectal injections, starting with doses smaller than would produce the reaction and gradually increasing the dose until the patient can partake of the desired amount of food without exhibiting any of the usual unpleasant symptoms. A good method of carrying out this procedure is by feeding the food in gelatin capsules.

Based on the results obtained from the injection of the pollen extracts, the most plausible and practical method, as carried out by some, is to prepare an aqueous extract of the food which would contain the proteid, and injecting this subcutaneously, starting with small doses, or by precipitating and desiccating the proteid and injecting the required amount of this in solution.

Unfortunately, however, none of these methods have proven entirely satisfactory, as quite a percentage of patients appear absolutely refractory, no matter what form of treatment is instituted.

Another use for the anaphylactic phenomena has been developed somewhat analogous to the precipitin test for the identification of blood, seminal, and other stains. Minot and Leclerc have shown that guinea-pigs, which have been sensitized by injections of human semen, suffer from symptoms of anaphylaxis on subsequent test injections. This has been proven specific, as other similarly sensitized guinea-pigs do not react to the injection of a testicular extract of other animals, or to the injection of human serum. Dale, also, has recommended the anaphylactic reaction for the identification of protein substances, such as blood-stains, *in vitro*, by the use of the graphic method with excised uterine muscles. It was found that guinea-pigs sensitized with human

serum react much better with human serum than that of the higher apes, and not at all with the serum of other species, such as the dog, ox, or fowl.

Discussion.—Considering the various instances cited for the application of the anaphylactic phenomenon, it appears evident that it is one of the most valuable diagnostic agents we have at present, and while it is not the dangerous reaction it was once thought, its absolute control must be acquired and more must be known about it before it can be employed for every-day clinical purposes, to advantage, in all instances.

REPRINTS OF PUBLICATIONS FROM THE RESEARCH LABORATORY, PARKE, DAVIS & CO., DETROIT, MICH.

The present system of collecting reprints of articles published from the Research Laboratory was begun in 1912. Reprints of the following articles published subsequent to that time are available and will be sent upon request. The publications marked (*) are no longer available.

1. On the Administration of Diphtheria Toxin in a Collodion Sac. By E. C. L. Miller. (*Journal of Infectious Diseases,* Vol. 8, January, 1911, pp. 50-65.)

2. A Further Contribution to Our Knowledge of Insecticides—Fumigants. By Chas. T. McClintock, H. C. Hamilton and F. B. Lowe. (*Journal of the American Public Health Association,* Vol. 1, April, 1911, pp. 227-238.)

3. Duboisia Hopwoodii—A Histological Study. By Oliver A. Farwell. (Reprinted from *Merck's Report,* Vol. 20, May 1, 1911.)

*4. Etiology of Canine Distemper. By Newell S. Ferry. (*Journal of Infectious Diseases,* Vol. 8, June, 1911, pp. 399-420.)

*5. The Resistance of Smallpox Vaccine to the Coal-tar Disinfectants. By Chas. T. McClintock and Newell S. Ferry. (*Journal of the American Public Health Association,* Vol. 1, June, 1911, pp. 418-419.)

*6. Production of Immunity with Over-Neutralized Diphtheria Toxin. By Chas. T. McClintock and Newell S. Ferry. (*Abdruck Aus Dem Centralblatt für Bakteriologie, Parasitenkunde und Infectionskrankheiten,* Abt. 1, Originale, Bd. 59, July 15, 1911, pp. 456-464.)

7. Soaps from Different Glycerides—Their Germicidal and Insecticidal Values Alone and Associated with Active Agents. By H. C. Hamilton. (*Journal of Industrial and Engineering Chemistry,* Vol. 3, August, 1911, pp. 582-584.)

*8. The Sleepy Grass of New Mexico: A Histological Study. By Oliver A. Farwell. (*Merck's Report,* Vol. 20, October, 1911, pp. 271-273.)

*9. Some Observations on the Physiological Action of Sleepy Grass. By A. W. Lescohier. (*Merck's Report,* Vol. 20, October, 1911, pp. 271-275.)

*10. An Investigation of the Depressor Action of Pituitary Extracts. By Carey P. McCord. (*Archives of Internal Medicine,* Vol. 8, November, 1911, pp. 609-620.)

11. The Physiology of the Pituitary Gland and the Actions of Its Extracts. By Carl J. Wiggers. (*American Journal of Medical Sciences,* Vol. 141, April, 1911, pp. 502-515.)

12. A Physiological Investigation of the Treatment of Hemoptysis. By Carl J. Wiggers. (*Archives of Internal Medicine,* Vol. 8, 1911, pp. 17-38.)

13. Notes on Catgut Sterilization: A Preliminary Report. By Willard H. Hutchings. (*Annals of Surgery,* Vol. 54, July, 1911, pp. 693-695.)

14. The Relations of Pyogenic Microorganisms to the Etiology and Treatment of Skin Diseases. By Henry Rockwell Varney. (*Ohio State Medical Journal,* December, 1911.)

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16. Serum Treatment of Hemorrhage and Blood Dyscrasias. By A. W. Lescohier. (*New York Medical Journal,* Vol. 95, February 3, 1912, pp. 223-229.)

*17. Further Studies on the Bacillus Bronchicanis, the Cause of Canine Distemper. By Newell S. Ferry. (*American Veterinary Review,* Vol. 41, April, 1912, pp. 77-79.)

18. The Pharmacopœial Requirements for Cannabis Sativa. By H. C. Hamilton. (*Journal of the American Pharmaceutical Association,* Vol. 1, March, 1912, pp. 200-203.)

19. The Heart Tonic Unit. By H. C. Hamilton. (*American Journal of Pharmacy,* Vol. 84, March, 1912, pp. 97-103.)

* 20. Studies on the Etiology of Equine Influenza. By Newell S. Ferry. (*Veterinary Journal* (London), Vol. 19, April, 1912, pp. 185-197.)

* 21. A Method for the Bacteriological Standardization of Disinfectants. By Tatsuzo Ohno and H. C. Hamilton. (*American Journal of Public Health,* Vol. 2, May, 1912, pp. 331-338.)

22. Physiological Testing. By E. M. Houghton. (*American Druggist,* July and September, 1911, and January and April, 1912.)

23. Bacillus Bronchisepticus (Bronchicanis): The Cause of Distemper in Dogs and a Similar Disease in Other Animals. By Newell S. Ferry. (*Veterinary Journal* (London), Vol. 19, July, 1912, pp. 376-391.)

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28. Pituitary Extracts in Obstetrics and Gynecology. By A. W. Lescohier and O. E. Closson. (*Journal of the Michigan State Medical Society,* Vol. 11, October, 1912, pp. 650-657.)

29. Biological Products—Veterinary. By Robert H. Wilson. (*American Veterinary Review,* Vol. 41, September, 1912, pp. 668-681.)

30. The Isolation and Cultural Characteristics of Bacillus Acne. By Edwin M. Stanton. (*Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten,* Original, Bd. 66, Heft 5/7, 1912, pp. 386-389.)

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34. The Iodine Content of the Small, Medium and Large Thyroid Glands of Sheep, Beef and Hogs. By T. B. Aldrich. (Original Communications, Eighth International Congress of Applied Chemistry, Vol. 19, 1912, pp. 9-14.)

35. Studies on the Virus of Hog Cholera. By Walter E. King and Robert H. Wilson. (*Zeitschrift für Immunitatsforschung und Experimentelle Therapie,* Bd. 16, Heft 3, 1913, pp. 367-376.)

*36. On the Cultivation of the Treponema Pallidum (Spirochæta Pallida). By F. W. Baeslack. (*Journal of Infectious Diseases,* Vol. 12, Jan., 1913, pp. 55-67.)

*37. Studies on the Gonococcus, I. By Carl C. Warden. (*Journal of Infectious Diseases,* Vol. 12, Jan., 1913, pp. 93-105.)

38. Studies on the Virus of Hog Cholera. By Walter E. King, F. W. Baeslack and George L. Hoffmann. (*Journal of Infectious Diseases,* Vol. 12, March, 1913, pp. 206-235.)

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40. Drug Influence on Extrasystoles of the Mammalian Heart. By Carey P. McCord. (*Interstate Medical Journal,* Vol. 19, Oct., 1912, pp. 870-880.)

41. The Employment of Protective Enzymes of the Blood as a Means of Extracorporeal Diagnosis. I.—Sero-Diagnosis of Pregnancy. By Carey P. McCord. (*Surgery, Gynecology and Obstetrics,* Vol. 16, April, 1913, pp. 418-421.)

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43. On Feeding Young White Rats the Posterior and the Anterior Parts of the Pituitary Gland. By T. B. Aldrich. (*American Journal of Physiology,* Vol. 31, Nov., 1912, pp. 94-101.)

44. The Rationale of the Use of Adrenalin in the Treatment of Asthma. By Carey P. McCord. (*Medical Record,* Vol. 83, March 8, 1913, pp. 431-432.)

45. Standardization of Disinfectants: Some Suggested Modifications. By H. C. Hamilton and T. Ohno. (*American Journal of Public Health,* Vol. 3, June, 1913, pp. 582-588.)

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49. On Crystalline Kombe-Strophanthin. By D. H. Brauns and O. E. Closson. (*Journal of the American Pharmaceutical Association,* May, June and July, 1913, Vol. 2.)

50. A Comparative Study of Antigens for the Wassermann Reaction. By H. R. Varney and F. W. Baeslack. (*Journal of the American Medical Association,* Vol. 61, Sept. 6, 1913, pp. 754-757.)

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64. Bacteriology and Control of Acute Infections in Laboratory Animals. By N. S. Ferry, Ph.B., M.D. (*Journal of Pathology and Bacteriology,* Vol. 18, 1914, pp. 445-455.)

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AMERICAN RECORDS OF DIOCTOPHYME RENALE.*

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Just recently Riley (1916) has compiled the record of American cases of the giant kidney worm, *Dioctophyme renale,* from the dog in connection with the publication of some new cases which came under Professor Riley's observation. On looking over my reprints I find two other records which were published in places where Professor Riley would hardly be likely to find them. In order to complete his list—if this does complete it—the two records noted are summarized here, and another case recorded.

Halsted (1909), in an article on transplantation of parathyroid glandules in dogs, covering work done at Baltimore, Maryland, notes in the case of one animal, an old dog, the following:

"*Autopsy.* Made and dictated by Dr. Hennington. Heart and lungs normal. On opening the peritoneal cavity an extravasation of blood into the omentum was observed and, on more complete exposure, a large round worm *(Eustrongylus gigas* or *Dioctophyme renale) (?),* 90 cm. long and 1 cm. in diameter, presented itself free in the peritoneal cavity in the neighborhood of the spleen. It was still alive, and on being placed in warm water executed slight movements. The intestinal peritoneum was thickened and granular looking. The parietal peritoneum presented in places small, indefinitely circumscribed, roughened areas. More careful examination of the omentum showed that the extravasated blood followed the ramifications of the blood vessels. The liver presented on its surface whitish nodules one to three millimeters in diameter. ***The surface of the spleen was slightly roughened. ***Kidneys quite normal in appearance."

When females of *D. renale* occur in the kidney of the dog, the eggs produced by the worms pass out in the urine. On the other hand, when these females occur in the peritoneal cavity, and Halsted's case is undoubtedly such a case, the eggs are passed to the peritoneal cavity, where they act as irritants and become attached to the peritoneal surfaces by small adhesions which are visible macroscopically as roughened areas. It is probable that

*Reprinted from the Journal of the American Veterinary Medical Association, December, 1916.

the roughening of the parietal and visceral peritoneum noted by Halsted was due to this cause.

The other record of *D. renale* from the United States is that of Baker (1916) and consists of remarks made before the Twentieth Annual Convention of the Indiana Veterinary Medical Association as follows:

"That suggests an interesting discovery that we made in our dissecting room a few days ago. We sent over to the dog pound for thirty-six dead dogs for the juniors to dissect. Floating free in the abdominal cavity of one was the longest specimen of the *Eustrongylus visceralis* I ever saw. It was about 5/8 of an inch in diameter and twenty-nine inches long and blood red. I measured it myself. Twenty-five years or more ago I found two on postmortem in the same place, in the abdominal cavity of a dog. One was about 12 inches long and the other was 14. The short one was 5/8 of an inch in diameter and the other was 3/4 of an inch in diameter and blood red. You will find it described in the books."

Baker's records undoubtedly deal with cases of *D. renale*. Riley has noted that not all of the published cases listed by him can be accepted. He very properly rejects all the human cases. Breeder's case must be listed as doubtful. The number of worms present, 21, and their length, 9 to 12 centimeters, is more suggestive of lumbricoid worms present in the abdominal cavity as the result of perforation of the intestine. The kidney lesions are puzzling. They are not the typical lesions due to *D. renale*, but in the absence of adequate data as to the worms themselves, it is perhaps as well to regard the case as unproved one way or another.

An unpublished case of the occurrence of *D. renale* has just come to my attention. While at Ann Arbor, Michigan, recently, Doctor La Rue of the University of Michigan showed me a large male of this species which had been collected at Ann Arbor about a year previous.

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STUDIES ON DERIVATIVES OF TRIHALOGENTERTIARY-BUTYL-ALCOHOLS.

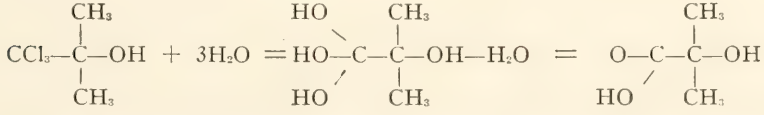
1. THE ACETIC ESTER OF TRIBROMOTERTIARY-BUTYL-ALCOHOL OR BROMETONE ACETIC ESTER.

BY T. B. ALDRICH AND C. P. BECKWITH.

(From the Research Laboratory of Parke, Davis & Company.)

The trichloro- and tribromotertiary-butyl-alcohols are most interesting compounds, both chemically and pharmacologically. The trichloro-compound, "Chloretone,"[1] has pronounced hypnotic, sedative and anesthetic properties, both local and general, and the same may be said of the tribromo-compound, "Brometone,"[2] which is considered to have more marked sedative but less pronounced hypnotic and anesthetic properties. Both compounds are sparingly soluble in water (chloretone 0.8% and brometone still less), but readily soluble in the organic solvents; both have a camphor-like odor and taste, are readily volatile in the air or with steam, and may be crystallized from dilute alcohol and obtained in the form of beautiful white crystals. Both compounds combine with water more or less firmly and in this respect resemble chloral, although the water is not chemically bound as in the latter substance to form a stable hydrate.

The three halogens attached to one carbon atom impart to these bodies properties different from those of the unsubstituted tertiary alcohols; indeed, as pointed out by Willgerodt,[3] they may be regarded as trihalides of *o-a*-hydroxyisobutyric acid, for they yield *a*-hydroxyisobutyric acid upon treatment with alkalies under suitable conditions, probably thus:



The trichlorotertiary-butyl-alcohol may be crystallized from warm moderately concentrated nitric acid without material decomposition. With care the tribromo-alcohol may be similarly crystallized, though with considerable decomposition. They are

[1]Chloretone and [2]brometone are the commercial names given the trichloro- and tribromotertiary-butyl-alcohols, respectively.

[3]*Ber.*, 15, 2305 (1882).

broken down by concentrated sulfuric acid and by moderately dilute caustic alkali solutions (5%). In general, they are chemically rather inert bodies, fairly resistant to anything short of destructive treatment. Willgerodt was unable to replace the chlorine of chloretone by alkyls.[1] It is interesting to note, however, that they form readily a series of esters of remarkable properties, and it is principally with these esters and related compounds that we are concerned in these articles.

It may be well to say in anticipation that one of the most striking properties of this series of esters is their relatively great stability- –a fact that was noted by one of us (C. P. B.) in 1903. At that time the salicylic ester of trichlorotertiary-butyl-alcohol was prepared and studied chemically and pharmacologically in the hope that it might be found to possess therapeutic value comparable with that of salol. The substance proved to be quite resistant to more or less heroic chemical treatment and to pass through the alimentary tract unchanged. Pharmacologically and clinically it appeared to be almost, if not quite, inert. These findings were not published at the time, and later the compound was produced in Germany and patented.[2]

An observation very useful in the purification of these esters is that all of them, so far prepared by us, seem to be quite unaffected by gentle warming with caustic alkali solutions of 5-10%, while the uncombined alcohols themselves are broken down and readily removed by this treatment.

In a former article one of us[3] obtained by acetylating trichloro-tertiary-butyl-alcohol with acetic anhydride and anhydrous sodium acetate in the usual manner, an ester to which the name acetyl chloretone was given. It has since been noted that this ester had already been prepared and briefly described by Willgerodt[4] under the unusual name, however, of acetyl-oxy-isobutyric-acid-trichloride.

The present article is concerned chiefly with the preparation and properties of a like compound of tribromotertiary-butyl-alcohol, the brometone acetic ester being formed similarly to the chloretone ester according to the following equation:

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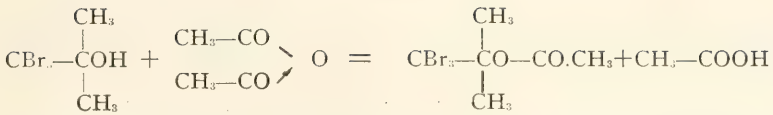
[1] *J. prakt. Chem.*, 39, 283-289 (1889).
[2] R. Wolffenstein, D. R. P. No. 267,381.
[3] T. B. A., This Journal, 37, 2726 (1915).
[4] *J. prakt. Chem.*, 39, 283-289 (1889).



Preparation.—(*a*) One part of brometone is boiled with two parts of acetic anhydride and one part of anhydrous sodium acetate for two hours, using a reflux condenser. During the heating, the mixture becomes slightly colored, due, no doubt, to the splitting off of bromine or bromine compounds. On cooling, the mixture solidifies, and on adding water and allowing to stand for some time two layers form, the upper being water, acid, sodium acetate, etc., the lower containing the product desired.

The upper layer is decanted as closely as possible, the residue warmed gently with an excess of caustic soda solution, and after standing some time extracted with ether. The ethereal extract is washed thoroughly, then filtered, and the ether allowed to evaporate. The residue left is distilled with steam. A colorless oil passes over, having an odor very similar to but not so pronounced as that of chloretone acetic ester. It is collected with ether, filtered, and the ether allowed to evaporate. Yield of nearly 50%. It colored slightly yellow on standing, and solidified. When purified by recrystallizing from alcohol it is white and melts at 43-44° (uncorr.).

(*b*) The following method of preparation is simpler and gives nearly a quantitative yield:

Dissolve two parts of brometone in four parts of glacial acetic acid and to this solution add one and a half parts of acetyl bromide or one part of the chloride. The mixture becomes warm and fumes of the halogen acid are given off. After the reaction has proceeded at room temperature for some time, the flask is heated on the steam bath for two hours and then allowed to stand overnight. Dilute caustic alkali is then added and the mixture warmed to decompose any excess of acetyl haloid or brometone. The ester settles then to the bottom of the vessel as an oil. The water, etc., above the oil is decanted and the latter washed several times with water. This oil on cooling strongly, and especially when inoculated with a crystal of the ester, solidifies at once in crystalline form. It may be recrystallized from alcohol. The yield is nearly quantitative.

The preparation may also be carried out without the use of

glacial acetic acid as a diluent. In this case the acetyl haloid is poured directly on the brometone, but considerable heat is evolved and it is advisable to provide for adequate cooling. After the reaction is ended, the vessel is heated on the steam bath until very little acid is given off. The compound is then treated as in the other cases.

Whichever method is used, economy of acetylating reagents is served, no doubt, by preliminary drying of the brometone as far as practicable. On the other hand, attempts to dry brometone thoroughly by most of the usual methods entail loss of this substance through volatilization, decomposition, or otherwise. For use in the reaction under discussion, a few days' standing in a desiccator over calcium chloride will suffice. (Sulfuric acid should not be used as a drying agent, since it absorbs and decomposes brometone vapor.) In fact, save that a larger proportion of the acetylating reagent is required, there is no objection to using ordinary crystallized brometone without preliminary drying.

Bromine determinations (Carius) carried out with a product recrystallized several times from moderately strong alcohol and melting at 43-44°, gave the following results:

0.4672, 0.2869, 0.2697, and 0.2584 g. gave 0.3161, 0.1985, 0.1841, and 0.1775 g. Br. Calc. for $C_8H_5O_2Br_3$: 67.99% Br. Found: 67.66, 69.20, 68.26, and 68.69%.

Leaving out the second value the results are sufficiently near, especially when the method of preparation is considered, to characterize the compound, without the necessity of making a combustion analysis, as the brometone acetic ester.

Properties.—The compound is extremely soluble in strong alcohol, acetone, chloroform, ether, glacial acetic acid, benzene, etc., insoluble in water. The alcoholic solution is precipitated by water.

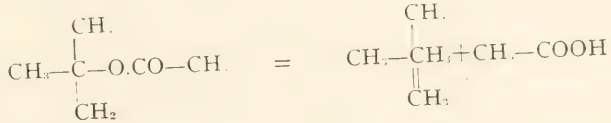
When boiled with water for some time (29 hrs.), using a reflux condenser, a portion of the substance in the form of an oil, estimated at 0.5 of that taken originally, is found undecomposed. This oil on cooling has a tendency to crystallize. The supernatant liquid is strongly acid toward litmus and gives a heavy yellowish precipitate with $AgNO_3$, soluble, though not quickly, in an excess of strong ammonia. There is no evidence of the alcohol in the condenser, as was the case when the chloretone ester was treated similarly. No brometone or other body is

thrown out by diluting the supernatant liquid with water. Further boiling for 21 hours completely decomposes the remainder of the oil. Probably the brometone ester is saponified and then the brometone decomposed. When brometone acetic ester is boiled with water, to which H_2SO_4 has been added, decomposition takes place in about the same time as when water alone is used.

Three grams of the substance were placed in a pressure tube with 10 cc. of H_2O and heated for three hours at 160°. There was a slight pressure on opening the tube, a combustible gas was given off, and an oil insoluble in H_2O had formed which did not solidify on cooling in ice water. On reheating the resealed tube for several hours at 170° complete decomposition of the oil-like substance had taken place, the homogeneous liquid had become yellowish, had a strong acid reaction toward litmus and showed the presence of large quantities of hydrobromic acid and of traces of acetic acid. By diluting with water, brometone was not thrown out. Partial carbonization had occurred.

Although saponification takes place slowly by boiling with water or water and dilute sulfuric acid, it takes place very rapidly when the ester is heated with an excess (three or four times its volume) of concentrated nitric acid. In fact, the brometone ester conducts itself in general toward hot nitric acid the same as the chloretone ester, except that the brometone which is produced at first readily undergoes further decomposition if heat is applied too long. In saponifying the brometone ester the procedure is as follows: Heat with the acid over a free flame until the ester dissolves. Then cool under running water. If a cloudiness appears, the saponification is not far enough advanced. Heat gently until on cooling the solution remains clear, then dilute with an excess of water. The brometone is precipitated and may be recrystallized from dilute alcohol.

Here again, as with chloretone acetic ester, it is to be noted that saponification may occur with reproduction of the original acid and alcohol, while the usual rule with tertiary alcohols is that an unsaturated hydrocarbon appears instead of the alcohol, thus:



Brometone acetic ester volatilizes slowly, much more slowly than brometone. Placed on a watch glass under a funnel at summer temperature for 12 days, it lost 23% of its weight. In the incubator for 7 days a sample lost 12.5%. Under like conditions the loss of brometone is much greater.

The following preliminary data relative to the pharmacological action of brometone acetic ester as compared with chloretone were kindly furnished by our associate, Mr. L. W. Rowe:

"The toxicity of this preparation was determined by intraperitoneal injection into guinea-pigs of an olive oil solution and the minimum fatal dose was found to be 0.5 g. per kg. body weight, that of chloretone being 0.15 g. per kg. body weight.

"Because of the practically complete insolubility of the product in water, it was impossible to determine the irritation, if any, which was produced by hypodermic injection. When an olive oil solution was used, some irritation was produced after some hours due to the very slow absorption. When a strongly alcoholic solution was used, irritation was produced immediately by the alcohol. The product itself probably does not possess very irritating properties.

"Concerning the anesthetic and sedative action of this drug we can say that as the dose approaches very near the toxic dose, an anesthetic action is observed several hours after administration. The slow action is no doubt due to the very slow absorption of the drug. The anesthetic action, which may be partially due to the toxicity of the drug, is certainly not as strong or as rapidly evidenced as that produced by chloretone.

"The action of brometone acetic ester upon the laid-bare frog's heart is not as strong as is that of chloretone.

"The blood pressure of an anesthetized dog was somewhat lowered after the intravenous injection of rather large amounts of this drug (4 cc. of a 2% solution in 50% alcohol). A control injection of the same amount of alcohol alone failed to produce as marked a reaction.

"In summarizing these data it seems that brometone acetic ester is somewhat similar to chloretone in its pharmacological action, but that the action of the former is not nearly as strong or as quickly evidenced. The latter fact can partially be accounted for by the extreme insolubility in water of the brometone acetic ester."

Further pharmacological studies are being made with these esters in comparison with chloretone and brometone.

Owing to delay and confusion in the mails from Germany, there has just reached us an article by R. Wolffenstein, A. Loewy and M. Bachstez on "Esters of Trichlorotertiary-butyl-alcohol and Their Pharmacology."[1] The details of their pharmacological findings are published in a separate article in a number of Schmiedeberg's Archiv. that has not yet come to hand. For the present, it will suffice to say that the results obtained by these gentlemen with the chloretone esters are, in most points, quite in accord with ours with the brometone esters in so far as we have followed parallel lines.

According to these authors the preparation of the esters is ordinarily easily carried out through the action of the acid chloride with or without the aid of tertiary bases, in fact from the acid and the alcohol in the presence of a condensing agent.

The following facts were observed by Wolffenstein relative to the esters of trichlorotertiary-butyl-alcohol: They are not as a rule split up in the body; they exhibit an unexpected action quite different from that of the alcohol from which they are prepared; and they exhibit poisonous properties, causing cramps. These cramps or convulsions begin to manifest themselves in chloretone acetic ester, increasing in the higher homologues and reaching the maximum as far as investigated in the ester of isovaleric acid.

It is further stated that the chloretone acetic ester, the one most thoroughly studied, shows less narcotic action but greater poisonous properties than chloretone.[2] The decrease in narcotic property and increase in poisonous property is shown much more plainly in the propionic ester and still more in the isovaleric ester. The isovaleric ester has no hypnotic action, but has, at least on rabbits, a toxic action causing convulsions.

The authors furthermore state that the only[3] known ester of trichlorotertiary-butyl-alcohol, up until the appearance of their article, is the acetic acid ester prepared first by Willgerodt under

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[1] *Ber.*, 48, 2035-43 (1916).

[2] According to the results of Aldrich relative to the toxicity of acetyl-chloretone (trichlorotertiary-butyl acetic ester) it was found (THIS JOURNAL, 37, 2722 (1915)) that "The toxicity of this acetyl-ester, when introduced subcutaneously into guinea pigs, is slightly less than that of Chloretone." Possibly Wolffenstein and his collaborators employed another method of administration, which would account for the different findings.

[3] Willgerodt prepared also the benzoic ester, *J. prakt. Chem.*, *loc. cit.*

the name acetyl-oxyisobuttersäure-trichloride. [Later by Aldrich under the name monoacetyl trichlorotertiary-butyl-alcohol (chloretone acetic ester).]

The following esters of chloretone were prepared by Wolffenstein: Propionic ester (yellow oil); isovaleric ester (oil); bromoisovaleric ester (oil); monochloroacetic ester (cryst.); trichloroacetic ester (cryst.); diethyl glycine ester (oil); dimethyl glycine ester (oil); piperidine acetyl ester (cryst.); allophanic ester (cryst.); acid malonic ester (cryst.); dibromocinnamic ester (cryst.); neutral malonic ester (cryst.).

SUMMARY.

The acetic ester of tribromotertiary-butyl-alcohol is most conveniently prepared through the interaction of acetyl chloride or bromide and the alcohol, or by means of acetic anhydride and anhydrous sodium acetate, in the usual way.

Prepared by either of these processes and recrystallized from alcohol, the purified substance melts at 43-44° (uncorr.).

Bromine determinations (Carius) gave results sufficiently near to characterize the compound as brometone acetic ester with the formula.

$$C_6H_5O_2Br_3 = CBr_3—\overset{\overset{CH_3}{|}}{C}.O.OC—CH_3.$$
$$\underset{CH_3}{|}$$

The compound is extremely soluble in the organic solvents, practically insoluble in water. It is not readily saponified by boiling with water or acidulated water, and when saponified the alcohol is decomposed still further. Although saponification takes place slowly by boiling with water or water and acid, it takes place very quickly when heated with an excess of moderately concentrated nitric acid. Like chloretone and brometone, though not quite so readily, the ester is volatile in the air and especially with steam. The pharmacological action is similar to that of chloretone and brometone although, presumably on account of its greater insolubility in water, its effects are less rapid and marked.

REPRINTS OF PUBLICATIONS FROM THE RESEARCH LABORATORY, PARKE, DAVIS & CO., DETROIT, MICH.

The present system of collecting reprints of articles published from the Research Laboratory was begun in 1912. Reprints of the following articles published subsequent to that time are available and will be sent upon request. The publications marked (*) are no longer available.

1. On the Administration of Diphtheria Toxin in a Collodion Sac. By E. C. L. Miller. (*Journal of Infectious Diseases,* Vol. 8, January, 1911, pp. 50-65.)

2. A Further Contribution to Our Knowledge of Insecticides—Fumigants. By Chas. T. McClintock, H. C. Hamilton and F. B. Lowe. (*Journal of the American Public Health Association,* Vol. 1, April, 1911, pp. 227-238.)

3. Duboisia Hopwoodii—A Histological Study. By Oliver A. Farwell. (Reprinted from *Merck's Report,* Vol. 20, May 1, 1911.)

*4. Etiology of Canine Distemper. By Newell S. Ferry. (*Journal of Infectious Diseases,* Vol. 8, June, 1911, pp. 399-420.)

*5. The Resistance of Smallpox Vaccine to the Coal-tar Disinfectants. By Chas. T. McClintock and Newell S. Ferry. (*Journal of the American Public Health Association,* Vol. 1, June, 1911, pp. 418-419.)

*6. Production of Immunity with Over-Neutralized Diphtheria Toxin. By Chas. T. McClintock and Newell S. Ferry. (*Abdruck Aus Dem Centralblatt für Bakteriologie, Parasitenkunde und Infectionskrankheiten,* Abt. 1, Originale, Bd. 59, July 15, 1911, pp. 456-464.)

7. Soaps from Different Glycerides—Their Germicidal and Insecticidal Values Alone and Associated with Active Agents. By H. C. Hamilton. (*Journal of Industrial and Engineering Chemistry,* Vol. 3, August, 1911, pp. 582-584.)

*8. The Sleepy Grass of New Mexico: A Histological Study. By Oliver A. Farwell. (*Merck's Report,* Vol. 20, October, 1911, pp. 271-273.)

*9. Some Observations on the Physiological Action of Sleepy Grass. By A. W. Lescohier. (*Merck's Report,* Vol. 20, October, 1911, pp. 271-275.)

*10. An Investigation of the Depressor Action of Pituitary Extracts. By Carey P. McCord. (*Archives of Internal Medicine,* Vol. 8, November, 1911, pp. 609-620.)

11. The Physiology of the Pituitary Gland and the Actions of Its Extracts. By Carl J. Wiggers. (*American Journal of Medical Sciences,* Vol. 141, April, 1911, pp. 502-515.)

12. A Physiological Investigation of the Treatment of Hemoptysis. By Carl J. Wiggers. (*Archives of Internal Medicine,* Vol. 8, 1911, pp. 17-38.)

13. Notes on Catgut Sterilization: A Preliminary Report. By Willard H. Hutchings. (*Annals of Surgery,* Vol. 54, July, 1911, pp. 693-695.)

14. The Relations of Pyogenic Microorganisms to the Etiology and Treatment of Skin Diseases. By Henry Rockwell Varney. (*Ohio State Medical Journal,* December, 1911.)

15. A Micrococcus with Unusual Characteristics as a Factor in a Resistant Dermatosis Resembling Acne Vulgaris. By Henry Rockwell Varney and L. T. Clark. (*Journal of Cutaneous Diseases,* Vol. 30, February, 1912, pp. 72-78.)

16. Serum Treatment of Hemorrhage and Blood Dyscrasias. By **A.** W. Lescohier. (*New York Medical Journal,* Vol. 95, February 3, 1912, pp. 223-229.)

*17. Further Studies on the Bacillus Bronchicanis, the Cause of Canine Distemper. By Newell S. Ferry. (*American Veterinary Review,* Vol. 41, April, 1912, pp. 77-79.)

18. The Pharmacopœial Requirements for Cannabis Sativa. By H. C. Hamilton. (*Journal of the American Pharmaceutical Association,* Vol. 1, March, 1912, pp. 200-203.)

19. The Heart Tonic Unit. By H. C. Hamilton. (*American Journal of Pharmacy,* Vol. 84, March, 1912, pp. 97-103.)

*20. Studies on the Etiology of Equine Influenza. By Newell S. Ferry. (*Veterinary Journal* (London), Vol. 19, April, 1912, pp. 185-197.)

*21. A Method for the Bacteriological Standardization of Disinfectants. By Tatsuzo Ohno and H. C. Hamilton. (*American Journal of Public Health,* Vol. 2, May, 1912, pp. 331-338.)

22. Physiological Testing. By E. M. Houghton. (*American Druggist,* July and September, 1911, and January and April, 1912.)

23. Bacillus Bronchisepticus (Bronchicanis) : The Cause of Distemper in Dogs and a Similar Disease in Other Animals. By Newell S. Ferry. (*Veterinary Journal* (London), Vol. 19, July, 1912, pp. 376-391.)

24. On Feeding Young Pups the Anterior Lobe of the Pituitary Gland. By T. B. Aldrich. (*American Journal of Physiology,* Vol. 30, July, 1912, pp. 352-357.)

25. A Practical Portable Incubator. By Newell S. Ferry. (*Abdruck Aus Dem Centralblatt für Bakteriologie, Parasitenkunde und Infectionskrankheiten,* Abt. 1, Original, Bd. 65, Heft 4/5, 1912, pp. 412-413.)

26. Tobacco Extracts: Their Comparative Values as Insecticides. By W. O. Hollister. (*Journal of Economic Entomology,* Vol 5, June, 1912, pp. 263-267.)

27. The Pharmacological Assay of Pituitary Preparations. By H. C. Hamilton. (*Journal of the American Pharmaceutical Association,* Vol. 1, October, 1912, pp. 1117-1119.)

28. Pituitary Extracts in Obstetrics and Gynecology. By A. W. Lescohier and O. E. Closson. (*Journal of the Michigan State Medical Society,* Vol. 11, October, 1912, pp. 650-657.)

29. Biological Products—Veterinary. By Robert H. Wilson. (*American Veterinary Review,* Vol. 41, September, 1912, pp. 668-681.)

30. The Isolation and Cultural Characteristics of Bacillus Acne. By Edwin M. Stanton. (*Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten,* Original, Bd. 66, Heft 5/7, 1912, pp. 386-389.)

31. Studies on Hog Cholera. By Walter E. King and Robert H. Wilson. (*Journal of Infectious Diseases,* Vol. 11, Nov., 1912, pp. 441-458.)

32. Studies on the Virus of Hog Cholera. By Walter E. King and F. W. Baeslack. (*Journal of Infectious Diseases,* Vol. 12, Jan., 1913, pp. 39-41.)

33. The Physiological Activity of Cannabis Sativa. By H. C. Hamilton, A. W. Lescohier and R. A. Perkins. (*Journal of the American Pharmaceutical Association,* Vol. 2, Jan., 1913, pp. 22-30.

34. The Iodine Content of the Small, Medium and Large Thyroid Glands of Sheep, Beef and Hogs. By T. B. Aldrich. (Original Communications, Eighth International Congress of Applied Chemistry, Vol. 19, 1912, pp. 9-14.)

35. Studies on the Virus of Hog Cholera. By Walter E. King and Robert H. Wilson. (*Zeitschrift für Immunitatsforschung und Experimentelle Therapie,* Bd. 16, Heft 3, 1913, pp. 367-376.)

*36. On the Cultivation of the Treponema Pallidum (Spirochæta Pallida). By F. W. Baeslack. (*Journal of Infectious Diseases,* Vol. 12, Jan., 1913, pp. 55-67.)

*37. Studies on the Gonococcus, I. By Carl C. Warden. (*Journal of Infectious Diseases,* Vol. 12, Jan., 1913, pp. 93-105.)

38. Studies on the Virus of Hog Cholera. By Walter E. King, F. W. Baeslack and George L. Hoffmann. (*Journal of Infectious Diseases,* Vol. 12, March, 1913, pp. 206-235.)

39. Bacillus Bronchisepticus—Its Relation to Canine Distemper. By N. S. Ferry. (*American Veterinary Review,* Vol. 43, April, 1913, pp. 16-30.)

40. Drug Influence on Extrasystoles of the Mammalian Heart. By Carey P. McCord. (*Interstate Medical Journal,* Vol. 19, Oct., 1912, pp. 870-880.)

*41. The Employment of Protective Enzymes of the Blood as a Means of Extracorporeal Diagnosis. I.—Sero-Diagnosis of Pregnancy. By Carey P. McCord. (*Surgery, Gynecology and Obstetrics,* Vol. 16, April, 1913, pp. 418-421.)

42. Tribromo-tert-Butyl Alcohol, $C_4H_7OBr_3$. By T. B. Aldrich. (*Journal of the American Chemical Society,* Vol. 33, March, 1911, pp. 386-388.)

43. On Feeding Young White Rats the Posterior and the Anterior Parts of the Pituitary Gland. By T. B. Aldrich. (*American Journal of Physiology,* Vol. 31, Nov., 1912, pp. 94-101.)

44. The Rationale of the Use of Adrenalin in the Treatment of Asthma. By Carey P. McCord. (*Medical Record,* Vol. 83, March 8, 1913, pp. 431-432.)

45. Standardization of Disinfectants: Some Suggested Modifications. By H. C. Hamilton and T. Ohno. (*American Journal of Public Health,* Vol. 3, June, 1913, pp. 582-588.)

46. Preventive Measures Against Equine Influenza Based on Its Bacteriology. By N. S. Ferry. (Report of the Proceedings of the United States Live Stock Association, December, 1912, p. 127.)

*47. Correcting Water. By H. C. Hamilton. (*Bulletin of Pharmacy,* Vol. 27, August, 1913, pp. 330-335.)

48. Duration of Immunity Following Small-pox Vaccination. By A. W. Lescohier. (*Journal of the American Medical Association,* Vol. 61, Aug. 16, 1913, page 487-490.)

49. On Crystalline Kombe-Strophanthin. By D. H. Brauns and O. E. Closson. (*Journal of the American Pharmaceutical Association,* May, June and July, 1913, Vol. 2.)

50. A Comparative Study of Antigens for the Wassermann Reaction. By H. R. Varney and F. W. Baeslack. (*Journal of the American Medical Association,* Vol. 61, Sept. 6, 1913, pp. 754-757.)

51. The Treatment of Tetanus. By Charles T. McClintock and Willard H. Hutchings. (*Journal of Infectious Diseases,* Vol. 13, Sept., 1913, pp. 309-320.)

52. Spirochæta Suis, Its Significance as a Pathogenic Organism, Studies on Hog Chlorea. By Walter E. King and George L. Hoffmann. (*Journal of Infectious Diseases,* Vol. 13, Nov., 1913, pp. 463-498.)

53. Time Recorder for Kymograph Tracings. By Oliver E. Closson. (*Journal of Pharmacology and Experimental Medicine*, Vol. 5, Jan., 1914, pp. 235-238.)

*54. U. S. P. Menstrua. By H. C. Hamilton. (*American Journal of Pharmacy*, Vol. 86, Feb., 1914, pp. 56-61.)

55. Numerical Variations of the White Blood Cells in Mice Inoculated with Transplantable Adenocarcinoma. By F. W. Baeslack. (*Zeitschrift für Immunitatsforschung und Experimentelle Therapie*, Bd. 20, Heft 5, 1914, pp. 421-435.)

56. A Study of the Germicidal Action of the Ultraviolet Rays. By E. M. Houghton and L. Davis. (*American Journal of Public Health*, Vol. 4, March, 1914, pp. 224-240.)

57. Some Phenomena Involved in the Life History of Spirochæta Suis—Studies on Hog Cholera. By W. E. King and R. H. Drake. (*The Journal of Infectious Diseases*, Vol. 14, March, 1914, pp. 246-250.)

*58. The Sterilization of Adrenalin Solutions. By L. W. Rowe. (*American Journal of Pharmacy*, Vol. 86, April, 1914, pp. 145-149.)

*59. Infection and Immunity: A Review. By N. S. Ferry, Ph.B., M.D. (*Journal of the American Pharmaceutical Association*, Vol. 3, April and May, 1914.)

*60. Disinfection—What Disinfectant is the Most Generally Applicable for Clinical, Surgical and Sanitary Purposes? By H. C. Hamilton. (*Therapeutic Gazette*, Vol. 38, May, 1914, pp. 311-315.)

61. Study of the Bacteriology of the Posterior Nasopharynx in Scarlatina. By N. S. Ferry, M.D. (*Medical Record*, Vol. 85, May 23, 1914, pp. 934-935.)

62. Some Experiences with Bacterial Vaccines in Scarlatina. By Guy L. Kiefer, M.D., D.P.H., and N. S. Ferry, M.D. (*Medical Record*, Vol. 85, May 23, 1914, p. 936.)

63. A Sero-enzyme Test for Syphilis. By F. W. Baeslack, M.A., M.D. (*The Urologic and Cutaneous Review*, Vol. 18, May, 1914, pp. 234-238.)

64. Bacteriology and Control of Acute Infections in Laboratory Animals. By N. S. Ferry, Ph.B., M.D. (*Journal of Pathology and Bacteriology*, Vol. 18, 1914, pp. 445-455.)

65. The Bacteriological Standardization of Disinfectants. By H. C. Hamilton and Tatsuzo Ohno. (*American Journal of Public Health*, Vol. 4, No. 6, p. 163.)

*66. The Pineal Gland in Relation to Somatic, Sexual and Mental Development. By Carey P. McCord, M.D. (*Journal of the American Medical Association*, Vol. 63, July 18, 1914, pp. 232-235.)

67. The Sero-enzyme Test for Syphilis. By F. W. Baeslack, M.D., M.A. (*Journal of the American Medical Association* Vol. 63, Aug. 15, 1914, pp. 559-563.)

68. A Case of Contagious Broncho-pneumonia Caused by Bacillus Coli Communis. By Edwin M. Stanton. (*American Veterinary Review*, Vol. 14, May, 1914, pp. 233-235.)

69. Local Anesthetics—Some Comparative Physiological Reactions. By Oliver E. Closson. (*Journal of the Michigan State Medical Society*, Vol. 13, Oct., 1914, pp. 587-597.)

*70. Potassium Tellurite as an Indicator of Microbial Life. By Walter E. King and Lewis Davis. (*American Journal of Public Health*, Vol. 4, Oct., 1914, pp. 917-932.)

71. Further Studies with Reference to Spirochetes Observed in Swine —Studies on Hog Cholera. By Walter E. King, Raymond H. Drake, and Geo. L. Hoffmann. (*Zeitschrift für Immunitätsforschung und Experimentelle Therapie,* Vol. 22, 1914, pp. 347-371.)

72. The Pharmacy of Adrenalin. By C. P. Beckwith. (*Journal of the American Pharmaceutical Association,* Vol. 3, November, 1914, pp. 1547-1554.)

73. A Study of the "Tellurite Reaction" with the Colon-typhoid Group and other Organisms. By Lewis Davis, S.M. (*Centralblatt für Bakteriologie Parasitenkunde und Infektionskrankheiten,* 75 Band, 1914, pp. 180-192.)

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76. Inoculation Experiment with Pure Culture of Spirochæta Hyos—Studies on Hog Cholera. By Walter E. King and Raymond H. Drake. (*Journal of Infectious Diseases,* Vol. 16, Jan., 1915, pp. 54-57.)

77. What is the Best End-Point of the Reaction in the Frog-Heart Method of Digitalis Assay? By H. C. Hamilton and L. W. Rowe. (*Journal of the American Pharmaceutical Association,* Vol. 4, January, 1915, pp. 108-112.)

78. The Glands of Internal Secretion and Their Importance as Therapeutic Agents. By Carey P. McCord. (*Journal of the American Pharmaceutical Association,* Vol. 4, March, 1915, pp. 293-297.)

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80. The Pineal Gland. By Carey P. McCord. (*Interstate Medical Journal,* Vol. 22, No. 4, April, 1915, pp. 354-370.)

81. The Filterability of Bacillus Bronchisepticus: With An Argument for a Uniform Method of Filtration. By N. S. Ferry. (*Journal of Pathology and Bacteriology,* Vol. 19, No. 4, April, 1915, pp. 488-493.)

82. The Pineal Gland in Relation to Somatic, Sexual and Mental Development. By Carey P. McCord. (*Journal of the American Medical Association,* Vol. 65, Aug. 7, 1915, pp. 517-520.)

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84. The Proper Time to Collect Sanguinaria. By O. A. Farwell. (*American Journal of Pharmacy,* Vol. 87, March, 1915, pp. 97-98.)

85. Notes on the Michigan Species of Polygonatum. By O. A. Farwell. (*Bulletin of the Torrey Botanical Club,* Vol. 42, May, 1915, pp. 247-258.)

86. Belladonna and Hyoscyamus. By O. A. Farwell. (*American Journal of Pharmacy,* March, 1915, pp. 99-101.)

87. Notes on Michigan Liliaceae. By O. A. Farwell. (*Bulletin of the Torrey Botanical Club,* Vol. 41, June 16, 1915, pp. 351-358.)

88. The Hemlock Spruce. By O. A. Farwell. (*Rhodora,* Vol. 17, No. 201, Sept., 1915, pp. 164-168.)

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90. An Effective Apparatus for Evaporating Aqueous Extracts by Means of a Current of Air. By T. B. Aldrich. (*Journal of Biological Chemistry,* Vol. 23, No. 1, Nov., 1915, pp. 255-259.)

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93. The Occurrence of Pituitrin and Epinephrin in Fetal Pituitary and Suprarenal Glands. By Carey P. McCord. (*Journal of Biological Chemistry,* Vol. 23, No. 2, Dec., 1915, pp. 435-438.)

94. The Principles of Vaccine Therapy and Why it Should be Used in the Treatment of Pyorrhea. By Geo. Bailey Harris and E. M. Stanton. (*The Dental Summary,* Vol. 57, Oct., 1915, pp. 792-799.)

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98. The Therapeutic Application of Ovarian Extract. By W. H. Morley, M.D. (*Journal of the Michigan State Medical Society,* Aug., 1916, Vol. 15, No. 8, pp. 372-377.)

99. Experimental Syphilis. By F. W. Baeslack, M.D. (*The Urologic and Cutaneous Review* (Technical Supplement), Vol. 4, No. 1, Jan., 1916, pp. 15-24.)

100. Pruritus Ani.—Preliminary Notes. By Louis J. Hirschman, M.S., F.A.C.S., and Herbert C. Ward, M.S. (*The Proctologist and Gastroenterologist,* Vol. 10, No. 3, Sept., 1916, pp. 193-198.)

101. The Ophthalmic Test for Glanders: With a Simplified Method of Procedure. By N. S. Ferry. (*Journal of the American Veterinary Medical Association,* Vol. 3, (N. S.) No. 1, October, 1916, pp. 41-46.)

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103. Pituitary Standardization. By H. C. Hamilton and L. W. Rowe. (*The Journal of Laboratory and Clinical Medicine,* Vol. 2, No. 2, Nov., 1916, pp. 120-129.)

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105. The Genus Hippochaete in North America, North of Mexico. By Oliver A. Farwell. (*Memoirs of the New York Botanical Garden,* Vol. 6, Aug., 1916, pp. 461-472.)

106. Further Studies on Hog Cholera with Reference to Spirochæta Hyos. By Walter E. King and R. H. Drake. (*Journal of the American Veterinary Medical Association,* Vol. 3, No. 3 (N. S.), Dec., 1916, pp. 168-188.)

107. Trichlor-Tertiarybutyl Alcohol Anesthesia. By L. W. Rowe. (*Journal of Pharmacology and Therapeutics,* Vol. 9, No. 2, Nov., 1916, pp. 107-112.)

108. The Phenomenon of Anaphylaxis: Its Clinical Significance and Practical Utilization. By N. S. Ferry. (*Therapeutic Gazette,* Vol. 32 (3d Series), No. 12, Dec., 1916, pp. 843-848.)

109. American Records of Dioctophyme Renale. By Maurice C. Hall. (*Journal of the American Veterinary Medical Association,* Vol. 3, No. 3 (N. S.), Dec., 1916, pp. 370-371.)

110. Studies on Derivatives of Trihalogentertiary-Butyl-Alcohols. By T. B. Aldrich and C. P. Beckwith. (*Journal of the American Chemical Society,* Vol. 38, No. 12, Dec., 1916, pp. 2740-2746.)

CONTRIBUTIONS TO THE BOTANY OF MICHIGAN NO. 14.

BY OLIVER ATKINS FARWELL.
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MICHIGAN NOVELTIES.

When any botanical field is, for the first time, subjected to an intensive study, it will produce a number of new and interesting forms, at least to the student of systematic botany. During my field work in and around Rochester, Oakland Co., Michigan, in 1914, a number of such were discovered, some of which form the basis of this paper. Two species, *Mitella diphylla* and *Apocynum Farwellii,* evidently in that condition which DeVries calls "Mutable," may be mentioned as especially illustrating one way of producing what he terms "elementary species." This is by way of suppression or obliteration of some part of the plant body. That the former species is in a mutable condition is more apparent as two variations have already been described and named, which are not due to a suppression or reduction of any part of the plant body.

The variations already named include forms with a third leaf on the scape situated between the normally opposite nearly similar leaves and the inflorescence and another wherein the leaves are long petioled. The form I have found has but one leaf on the scape. Here one of the normally opposite leaves has been suppressed; the species wherever it grows is found in large patches, and in such patches where this form with one leaf occurs, one will always find a series of individual plants that will show a complete gradation from the normal to this form; that is, with one leaf gradually getting smaller, while the other remains normal, until the blade has entirely disappeared and then a similar shortening of the petiole until there is no evidence on the surface of the scape of a second leaf. I have seen plants in which the leaf blade had been reduced to the size of a minute bract, perhaps 1-16 of an inch in length, and others on which the only indication of a second leaf was a small mucro of similar length and one that even had a flower opposite. Evidently the flower was of axial origin, but there was no indication whatever of the subtending leaf. It

may also be remarked that an axillary flower is quite an unusual condition in this species.

The other plant is one of our dogbanes. When first collected I had referred it to *Apocynum pubescens* R. Br., but Dr. E. L. Greene, of Washington, D. C., considered it distinct and has named it *Apocynum Farwellii.* It is common at Detroit and probably throughout lower Michigan. The typical form is glabrous and glaucous below pubescent on the upper parts as well as on the under side of the leaves; the flowers are small, a line in length, white, the lanceolate calyx lobes nearly as long as the corolla.

At Rochester, Oakland Co., there is a form that is glabrous and glaucous throughout. Both of these forms typically have a normal opposite phyllotaxis and the stems are more or less obtusely four angular. Each has a form that shows a triangular stem with leaves in whorls of three, and the glabrous form has also shown a state wherein the leaves are partly alternate, partly opposite, and partly subverticillate. One leaf of the three is occasionally wedge-shaped with a large indenture at the apex, as though a wedge-shaped section had been cut out, a transition towards four leaves to a whorl. Some of the branches are divided to the middle, the lower part being triangular with leaves in three, while each of the upper divisions are normal with the normal opposite phyllotaxis. These facts would seem to indicate a coalescence of two stems or plants, the condition known as fasciation. In a coalescence of two plants one might naturally expect a four-angular stem with, possibly, an intermediate angle or rib on each side and four leaves in a whorl; but the triangular stems and verticills of three leaves seem to indicate a reduction or suppression of a part of the stem and its accompanying leaf.

It might, incidentally, be remarked here that if the intensive studies of the American flora continue to be as productive in the future as they have been in the past, then in a very short period our manuals will necessarily be greatly restricted in range if all the forms are to be included and the manual maintained at a convenient size and within economical limits. Probably the time is not far distant when there will be a manual of botany for each State of the Union, each of which will include all the known forms of the region it covers. Some of the States, not Michigan,

however, are already supplied with their local manuals. I have considered preparing such a work, but present duties and obligations will not permit. Some one should, however, take up the work and carry it on to an early completion.

Corylus Americana, Walt. Var. altior N. Var.

Taller (15 to 18 feet) than the species. The involucre remaining erect over the nut, simulating a tube, the pubescence being generally thinner and shorter, and the glandular setae fewer, rarely absent.

The fruit is fairly well represented by Plate XIII, fig. 9, in the Department of Agriculture Bulletin on Nut Culture in the United States, 1896. Farwell, No. 2822, July 7, 1912, in swamps at Algonac; No. 3154, Sept. 8, 1912, in swamps at Algonac; No. 3266, Oct. 27, 1912, on dry hills at Stoney Creek.

Hepatica Hepatica (Lin.) Karst. var. albiflora (Raf.) N. Comb.

Hepatica alba Mill Dict. No. 3, 1768.

Hepatica triloba var. *albiflora* Raf. Medical Flora 1,239, 1828.

Hepatica triloba var. *alba*, Hort.; K. C. Davis in Bailey Cyclo. Amer. Hort. 2, 730, 1900.

Anemone Hepatica L. f. *alba* Mill.; Hegi Illus. Fl. Mit. Euro. 3, 529, 1913.

Flowers white or white flushed with a pale-bluish tinge. Rich woods, Farwell No. 3c June 1, 1883, from the Keweenaw Peninsula; No. 3593, April 6, 1914, from Rochester.

Hepatica Hepatica (L.) Karst. var. purpurea, N. Var.

Flowers purple. Rich woods. Farwell, No. 3595, April 16, 1914, from Rochester; No. 3d, June 1, 1883, from the Keweenaw Peninsula.

Hepatica Hepatica (L.) Karst. var. vulgaris (Mill) N. Com.

Hepatica vulgaris Mill. Dict. No. 4, 1768.

Anemone Hepatica L. f. *rosea* Neumann; Hegi Illus. Fl. Mit. Euro. 3, 529, 1913.

Flowers pink or white and streaked with pink. Rich woods. Farwell, No. 3b June 1, 1883, from the Keweenaw Peninsula; No. 3592, April 16, 1914, near Rochester.

Hepatica Hepatica (Lin.) Karst. var. parviflora (Raf.) N. Comb.

Hepatica triloba var. *parviflora* Raf. Med. Fl. 1, 239, 1828.

Flowers blue as in the specific type, but only half as large; sepals usually shorter than the involucre. Farwell, No. 3, June 1, 1883, Keweenaw Peninsula.

Ranunculus Michiganensis, N. Sp.

Similar to R. abortivus but coarser in all its parts and more succulent. Radicle leaves long-petioled, orbicular or reniform, cordate, with a deep and narrow or broad and shallow sinus often 2½ inches in diameter or more, coarsely crenate, some three-divided, the divisions stalked; lower cauline leaves often long petioled and three-divided, the divisions long-stalked, both gradually reduced until in the uppermost, leaves and divisions are sessile; the lateral divisions cuneate-obovate, two-parted, the larger three-lobed, crenate; the middle division lanceolate often 3 inches long, mostly entire or few toothed. Swamp lands near Rochester, Michigan. Farwell, No. 3627, May 17, 1914.

This may be the *R. abortivus* var. *eucyclus* Fernald, but it is more succulent than R. *abortivus,* while that variety is said to be not so succulent.

Mitella diphylla, Lin. Var. monophylla, N. Var.

Differs from the species in having but one leaf upon the stem. Farwell, No. 3629, May 15, 1914, near Rochester, Michigan.

Apocynum Farwellii, E. L. Greene, Pittonia.

A pubescent species that I had collected at Detroit July 7, 1893, and distributed as *A. pubescens* under the No. 1263a. It is quite common about Detroit.

Closely related to *A. hypericifolium* but has differently shaped leaves.

Apocynum Farwellii, E. L. Greene f. verticillare, N. Form.

Differs from the species in having the leaves in whorls of threes instead of opposite. Farwell, No. 3684, June 20, 1914.

Apocynum Farwellii, E. L. Greene var. glaucum, N. Var.

Differs from the species in having the entire plant glaucous and glabrous. In moist ground on thinly wooded hillsides at Rochester. Farwell, No. 3815, August 9, 1914.

Apocynum Farwellii, E. L. Greene var. glaucum f. ternarium, N. Form.

Differs from the var. *glaucum* in having the leaves in threes

instead of opposite. Rich, moist grounds on thinly wooded hillsides at Rochester. Farwell, No. 3724, July 19, 1914.

Apocynum Farwellii, E. L. Greene var. glaucum, f. anomalum, N. Form.

Differs from the var. *glaucum* in having some of the leaves alternate, some opposite, and some verticillate or subverticillate. Moist grounds on thinly wooded hillsides at Rochester. Farwell, No. 3803, July 30, 1914.

Apocynum Milleri, Britton var. pauciflorum, N. Var.

Plant, low, six or eight inches in height, bushy, the cymes mostly reduced to one or two flowers. Hills near Rochester. Farwell, No. 3725½, July 19, 1914.

Eupatorium urticaefolium, Rich. var. trifolium, N. Var.

Differs from the species in having the leaves in threes instead of opposite. Occasional with the ordinary form. Farwell, No. 3843, Aug. 23, 1914.

Lacinaria scariosa (Lin.) Hill var. trilisioides, N. Var.

I collected at Rochester, Mich. (3838½), on August 23, 1914, a plant that will answer very closely to the variety *sphaeroidea* (Mx.) Farwell, but it differs in some essentials. Michaux described his *Liatris sphaeroidea* as with stipitate heads, but in this plant the heads terminate foliolose peduncles two inches long, as in the specific type, but the heads are several times as numerous as in that, making a rather close spike; the plant agrees with Michaux's description in all other respects. What I chiefly wish to bring to your notice, however, is the fact that the plant has a distinct vanilla-like odor as in *Trilisa odoratissima* Cass. of the Southern States, but not so pronounced. So far as I am aware no species of *Lacinaria* has this odor, so I give it the varietal name *trilisioides*.

Lacinaria cylindracea (Mx.) O. K. Var. solitaria (MacM.) N. Comb.

Liatris cylindracea Var. *solitaria* MacM.; Gray, Man. Ed. 7, 785, 1908.

This variety is described in the Manual as with one, slightly enlarged, terminal head. At Rochester I collected a plant (No. 3818) on August 9, 1914, that was of the usual height for the species but was more slender, the leaves shorter, proportionately narrower, with but one terminal head which was reduced in size,

the lower involucral bracts being foliaceous and nearly as long as the oblong head, only slightly narrowed at base. I have no doubt but that it belongs here.

Solidago patula, Muhl. Var. macra, N. Var.

Plant about 2 feet high, very slender and delicate, leaves very thin, and infloresence, a short, wand like thyrse, 2 inches long. The inflorescence is that of *S. uliginosa* and allied species, *i.e.*, a thyrse with puberulent peduncles and involucres, but the leaves and stem are those of *S. patula* in everything but stoutness; the lower cauline leaves have ovate or oval, finely serrate blades 5-7 inches long by 3-4 wide on broadly winged petioles 2-5 inches long; the upper are elliptical, oblong, or oval, 3 inches or less long and ¾ to ½ as wide, acute, tapering into a short but distinct winged petiole; all very rough on the upper surface, otherwise glabrous; heads 2-2½ lines high and nearly as broad; involucral bracts oblong, narrowed at the apex, but obtuse, ciliate; achenes pubescent. Rich woods at Rochester, Farwell, No. 3868, Sept. 17, 1914.

Aster puniceus, Lin. Var. monocephalus, N. Var.

Stem simple, terminated by a single head; otherwise as in the species. Rochester, Farwell, No. 3866, Sept. 7, 1914.

Aster puniceus, Lin. Var. albiflorus, N. Var.

Rays white, otherwise as in the species. Rochester, Farwell, No. 3862, Sept. 7, 1914.

NOTES ON MISCELLANEOUS SUBJECTS, INCLUDING SOME NEW VARIETIES AND ADDITIONS TO THE MICHIGAN FLORA.

Ceanothus sanguineus, Ph. While sojourning in the Keweenaw Peninsula in the autumn of 1914, I came across a small *Ceanothus* in fruit with still a few leaves adhering to the shoots of the season. As I was there for the single purpose of making a search for this particular plant, I gathered a few specimens, notwithstanding the rather poor condition it was in. The fruiting racemes were not over 3 inches in length, the peduncles were rather stout and clustered together at what appeared to be the apex of the branch of the preceding year, or on the old wood, and below any of the leaves then remaining on the branches. The

leaves and racemes, as to length and position, agree very well with those of specimens of *Ceanothus sanguineus,* collected by Henderson in Oregon and now in the herbarium of Parke, Davis & Co.; also with *Ceanothus Oreganus* as shown on t. 5177 of the Botanical Magazine. There can be no doubt that the plant is either *Ceanothus sanguineus* or a new species closely allied to it. Rocky woods at Copper Harbor, Farwell, No. 3915½, Oct. 17, 1914.

Circaea Lutetiana L. Var. intermedia (Ehrh.), N. Comb.

Circaea intermedia, Ehrh. Beitr. 4, 42, 1790(?).

Circaea alpina Lin. Var. *intermedia* (Ehrh.) D. C., Prod. 3, 63, 1828. Farwell, No. 3814½, August 9, 1914. Rich woods near Rochester.

Circaea Lutetiana, L. Var. alpina (Lin.), N. Comb.

Circaea alpina Lin. Sp. Pl. 1, 9, 1753.

There is a perfect graduation between *C. Lutetiana* and *C. alpina* through the varieties *Canadensis* and *intermedia,* and it therefore seems best to unite the species. Indeed, this was accomplished by Sprengel in his edition of the Systema Vol. 1, 89 in 1825, and he did not there consider any of the forms worthy of being distinguished even by varietal names.

Vaccinium Pennsylvanicum, Lam. Var. myrtilloides (Mx.) Fernald.

This pubescent form of the common low blueberry is frequent at Algonac, where I collected it in 1914, and probably throughout the State. As indicated by Mr. Fernald it has been confused with *V. Canadense.* Farwell, No. 3638 and No. 3639, May 24, 1914.

AMELANCHIER.

So much has been written about these plants by botanists of all times that it seems hardly possible that there is anything left to write about. Confusion has arisen by a misinterpretation of descriptions or by actually ignoring them. The "Species" of Linnaeus differed widely from the "species" as understood today by such progressive botanists as are perusing an intensive study of systematic botany. The species listed in the Species Plantarum are not based upon type specimens as are species of today; that work is primarily an application of our binomial system to

plants that had been described by other botanists under the polynomial phrases according to the custom of old. It would not be surprising, therefore, if Linnaeus, under his broad concept of a species, included under a specific name some references which at the present time would not be considered as appertaining thereto, or that even Linnaeus, himself, upon a better knowledge of them, would accept as having been properly referred. It is not too much to say that the advanced student of systematic botany with his proclivities for intensive work now looks upon most species of Linnaeus as aggregates.

Linnaeus in the Species Plantarum Ed. 1, 478, 1753, described *Mespilus Canadensis* as follows:

"Mespilus inermis, foliis ovato-oblongis, glabris serratis, caule inermi.

Mispilus inermis, foliis subtus glabris obverse-ovatis, Gron. Virg. 54. Habitat in Virginia, Canada. 5."

In Systema Naturae XII, 343, 1767, as follows:

"M. inermis, fol. ovato-oblongis glabris serratis actuisisculis. *Tenera lanata; adultior nuda.*"

In the Species Plantarum both the description and the specific name indicate the northern plant that is glabrous from the beginning or very early becoming so. Even the reference to Gronovius is to a plant with glabrous leaves. The inference is that at the time of Gronovius there was in Virginia an Amelanchier with glabrous leaves, or, what is the same thing from a bibliographical aspect, Gronovius thought they were glabrous. In the Systema the description is of a plant, the young leaves of which are lanate. In other words, Linnaeus in the earlier publication described the smooth-leaved plant and later revised the description to include the one with tomentose leaves. As above indicated, considering his concept of a species, this is not at all surprising. The younger Linnaeus, noticing the discrepancy, raised the plant with tomentose leaves to specific rank as Mespilus Botryapium in Suppl. 255, 1781.

In any discussion of the application of the name *Crataegus spicata,* Lam. Enyc. 1, 84, 1783, one must not lose sight of the work of K. Koch. (Dendr. 1, 1823, 1869) who has demonstrated, as far as it is possible so to do, that the trees of their descendants, upon which LaMarck based his species, are still in existence in

certain parks in France and at the school of forestry there, and that these trees are what has later been known as *A. sanguinea* D. C. Furthermore, that the description of LaMarck fits these trees in all particulars. The evidence seems to be conclusive. The Michigan species of Amelanchier are as follows:

A. Canadensis (Lin.) Medic. Gesch. 79, 1793.

Mespilus Canadensis Lin. Sp. Pl. 478, 1753.

A. laevis Wiegand, Rhodora 14, 154, 1912.

A small tree the young leaves of which are very thin, brownish-purple, and generally glabrous from the beginning. Farwell, No. 48b, Aug. 10, 1883, and No. 53, Aug. 17, 1883, from the Keweenaw Peninsula; No. 1351½, May 6, 1893, from Belle Isle, and No. 48c, May 1, 1910, from Orion. This species in all its varieties has the young leaves thin and more or less brownish-purple. The type is a tree and the leaves are glabrous from the beginning. Moist woods and thickets.

A. Canadensis (Lin.) Medic. Var. rotundifolia, (Mx.) T. & G. Fl. N. A. 1, 473, 1840.

Crataegus spicata Lam. Encyl. 1, 84, 1783.

Mespilus Canadensis var. *rotundifolia* Mx. Fl. Bor. Am. 1, 291, 1803.

Amelanchier sanguinea (Ph.) D. C. Prod. 2, 633, 1825.

Amelanchier spicata (Lam.) K. Koch, Dendr. 1, 682, 1869.

A tall shrub or tree but not so large as the specific type. The leaves broader, often orbicular but acute. Thinly tomentose but glabrous or nearly so at time of flowering. Moist or dry woods and thickets. Farwell, No. 48, Aug 9, 1883; No. 48a, Aug. 10, 1883, and No. 50, Aug. 13, 1883, from the Keweenaw Peninsula; No. 49b, May 13, 1893, from Detroit; No. 48c, April 23, 1910, No. 2777, June 30, 1912, No. 3339, May 4, 1913, and No. 3354, May 11, 1913, from near Rochester; No. 3330, May 4, 1913, from Parkedale Farm.

Amelanchier Canadensis (Lin.) Medic. var. alnifolia (Nutt.) T. & G. Fl. N. A. 1, 473, 1840.

Aronia alnifolia Nutt Gen. 1, 306, 1818.

Amelanchier alnifolia Nutt. Roemer, Syn. Man. 3, 147, 1847.

Nuttall described his *Aronia alnifolia* as a low shrub 4 or 5 ft. in height. The name as at present understood is applied to a tree 30 ft. or thereabouts. All the Michigan plants have been referred

to A. florida and A. alnifolia has been eliminated from the eastern flora; but this is an error. True *A. alnifolia* is found here and is quite frequent in the Lake Superior region. A. florida is frequent around Rochester and probably at other places. Both have ovate to obicular leaves, but A. alnifolia has a bole and is therefore tree-like in habit if not in stature, while A. florida is densely caespitose, producing many stems; both have about the same height, 2-10 feet. It is customary to apply the name A. alnifolia to certain western *trees,* but why so is difficult to determine, as the species was originally described as a *shrub,* but whether tree-like or caespitose is not indicated. Farwell, No. 51, August 13, 1883, from the Keweenaw Peninsula; No. 907½, August 29, 1895, from near Orion.

Amelanchier Canadensis (Lin.) Medic. var. semiintegrifolia (Hook), N. Comb.

Amelanchier ovalis var. *semiintegrifolia* Hook Fl. Bor-Amer. 1, 202, 1834.

A florida Lindl. Bot. Reg. 19 pl. 1589, 1833.

A caespitose shrub 10 feet or less high with smaller leaves than those of the preceding though of the same general shape. Farwell, No. 51d, April 23, 1910, and No. 3332, May 4, 1913, from Rochester; No. 2779, June 30, 1912, from Parkedale Farm; No. 3335, May 4, 1913, from Stony Creek. *Amelanchier florida* table 8611 of Curtiss' Botanical Magazine is scarcely the same as Lindley's plate 1589. It has little in common with that outside of the leaf serratures. It agrees better with that of Lindley's A. sanguinea plate 1171 of the Botanical Register. It has the appearance of being the result of a cross between the two; it is briefly characterized as with the foliage of the former and the infloresence of the latter.

Amelanchier Canadensis (Lin.) Medic. Var. oligocarpa (Mx.) T. & G. Fl. No. Amer. 1, 474, 1840.

Mespilus Canadensis Lin. var. *oligocarpa,* Mx. Fl. Bor. Am. 1, 291, 1803.

Amelanchier Bartramiana Roemer Sys. Rosif. 145, 1847.

A low shrub not over 3 feet high forming dense clumps, having a habit much like that of *Rhamnus alnifolius* or *Juniperus communis,* var. *depressa.* The leaves are oval or elliptical, acute at apex and more or less cuneately acute at base, glabrous; flow-

ers one or two, rarely three, and apparently in the axils of the leaves. Swamps, Farwell, No. 52d, August 17, 1883, from the Keweenaw Peninsula.

Amelanchier Canadensis (Lin.) Medic. var. pauciflora, N. Var.

A low tree-like shrub, eight feet or less; leaves ovate, oblong, obovate, oval or elliptical, obtuse or acute and from cuneate to slightly cordate at base; all these variously shaped leaves are to be found on the same twig; they are glabrous or nearly so from the beginning; the flowers are in the axils of the leaves and single or from two to five in a lax and often a fastigiate raceme. Apparently this is a cross between the varieties *rotundifolia* and *oligocarpa*. Moist thickets and swamps. Farwell, No. 52, No. 52a, No. 52b, August 15, 1883, and 52c, August 17, 1883, from the Keweenaw Peninsula.

Amelanchier Botryapium (Lin. f.) Borkh. Handb. Forstb. 2, 1260, 1803 (?); D. C. Prod. 2, 632, 1825.

Mespilus Canadensis Lin. Syst. Nat. XII, 343, 1767, in large part, not Lin. Sp. Pl. 478, 1753.

Pyrus Botryapium Lin. f. Suppl. 255, 1781.

Mespilus Canadensis Lin. b. *cordata* Mx. Fl. Bor. Am. 1, 291, 1803.

A tree of generally drier situations than that of the preceding, but may be found in swampy places. Leaves never brownish-purple, thicker and stouter, ovate, acute, finely serrate, always densely tomentose at flowering time, becoming glabrous or glabrate only with age. Farwell, No. 49, August 10, 1883, from the Keweenaw Peninsula; No. 49c, April 23, 1910, from Rochester; Nos. 3317, 3327, and 3329, May 4, 1913, and No. 3613, July 20, 1913, from Parkedale Farm.

Amelanchier Botryapium (Lin. f.) Borkh. var. obovalis (Mx.), N. Comb.

Mespilus Canadensis Var. obovalis Mx. Fl. Bor. Am. 1, 291, 1803.

Amelanchier Canadensis B. *oblongifolia* T. & G. Fl. N. Am. 1, 473, 1840.

Amelanchier oblongifolia Roemer Nat. Syst. Rosifl. 147, 1847.

A shrub never attaining the size of a tree; leaves often broader above the middle than below. Farwell, No. 49a, August

10, 1883, from the Keweenaw Peninsula, No. 1351⅓, May 6, 1893, from Belle Isle; No. 49d, April 23, 1910, from Rochester; No. 3333, May 4, 1913, from Parkedale Farm. Gronovius described his Mespilus with glabrous, "obverse-ovatis" leaves; he may have had this variety with mature leaves which would have been glabrous or glabrate.

Amelanchier Botryapium (Lin. f.) Borkh. Var. micropetala (Robinson), N. Comb.

Amelanchier oblongifolia var *micropetala* Robinson, Rhodora, 10, 33, 1908.

A. humilis Wiegand, Rhodora, 14, 141, 1912.

A. stolonifera Wiegand, Rhodora, 14, 144, 1912.

A low stoloniferous shrub of rocky or sandy soils from half a foot to three or four in height. Farwell, No. 51a, August 13, 1883, and No. 3075, Aug. 22, 1912, from the Keweenaw Peninsula; No. 51b, July 16, 1905, from Island Lake; No. 51c, April 23, 1910, from Rochester; No. 3322, May 4, 1913, from Parkedale Farm.

Amelanchier Botryapium (Lin. f.) Borkh. Var. conferta, N. Var.

Small shrub similar to the preceding variety; raceme of 12 to 15 flowers about an inch in length, compact; calyx lobes five, with an inner row of five alternating and 10 petals in the interstices; petals equalling the calyx lobes or just overtopping them, about a line long, linear or spatulate and like the calyx lobes more or less woolly. Farwell, No. 3625, May 15, 1914, near Rochester.

Callistachya Virginica (Lin.) Raf. Variety lanceolata, N. Var.

Differs from the species in having narrowly lanceolate, acuminate leaves. Farwell, No. 1165, July 18, 1891, from Ypsilanti, No. 1165a, July 25, 1892, from Belle Isle, No. 2937, July 28, 1912, and No. 3834, Aug. 9, 1914, from Parkedale Farm, No. 2884, July 20, 1912, from Rochester.

The type of *Veronica Virginica* Lin. is the plant with broad (oval or elliptical) acute leaves. Both forms occur in Michigan, but the variety is much the commoner form. *Callistachya* Raf. in Med. Repos. New York, 5, 60, 1808, is the oldest name for this genus and is available under Articles 50 and 57 of the Vienna Rules. The former provides that no genus-name should be re-

jected, changed, or modified on account of an earlier homonym universally regarded as non-valid; and the latter that two generic names, differing only in the termination even if only by one letter, must be considered as distinct names. *Callistachys* Vent. (1803) and *Callistachya* Raf. (1808) must therefore be considered as distinct and valid generic names. Sir James Edward Smith used *Callistachya* in the same year as Rafinesque, but whether earlier or later I cannot say; but that is immaterial as it is a pure synonym of Callistachys, Vent., and, therefore, even if it antedates the use of the name of Rafinesque, the latter under Article 50 is valid. The speci s is of wide distribution, being found in Europe and Asia as well as in North America and has received many specific names. Two other forms may be worth recording here. One from Dahuria has blue flowers and very broad leaves and may be known as Callistachya Virginica (Lin.) Raf. Var. SIBIRICA (Lin.), Nov. Comb. *Veronica Sibirica* Lin. Sp. Pl. Ed. 1, 12, 1762. The other has purple flowers and is from Virginia, according to Pursh and others, and may be known as Callistachya Virginica (Lin.) Raf. Var. PURPUREA (Raf.), N. comb. *Eustachya purpurea* Raf. Am. Month. Magaz. 190, 1819; *Leptandra Virginica* (Lin.) Nutt Var. *purpurea* Ph. in Eaton & Wright N. Am. Bot. Ed. 8, 297, 1840.

PLANTAGO LANCEOLATA, Lin.

This is a very variable species, but four well-defined forms are recognizable. One with linear or linear-lanceolate, 3-5-ribbed, thinly hirsute blades, 3-5 inches long by 5 lines wide, on marginless petioles nearly or quite of their own length which together are about ¾ the length of the scape. Spikes oblong or nearly ovate. This apparently is the Linnean type. Another form is much taller and more robust, scapes about 20 inches long. The leaves are elliptical or oblong-lanceolate, over a foot in length by 1¾ inches in width, tapering into short winged petioles which with the blades are more or less boat-shaped; the blades are 7-nerved and cross-wrinkled; spikes cylindrical; this probably is Var. contorta, Guss. (*P. lanceolata* var. *altissima* Dcne.) A third form is somewhat similar but has smaller leaves (six inches or so long), not cross-wrinkled, and with the margined petioles flat; this appears to be var. irrigua, Dcne. A fourth form is found in sand or rocky grounds, and is not over 10 inches in height with

3-ribbed sessile or subsessile acute or accuminate leaves two or three inches long and not over 3 lines wide, generally densely hirsute; spikes short, ovate or sub-globular; this probably is Var. eriophylla, Webb. I have collected the forms from various places as follows:

PLANTAGO LANCEOLATA, Lin.

In fields and pastures No. 1138, June 13, 1891, Ypsilanti; No. 1138a, June 10, 1895, Belle Isle; No. 1138b, June 10, 1895, Mackinac Isle; No. 1138c, June 27, 1895, Keweenaw Peninsula; No. 3002, August 4, 1912, Parkedale Farm.

PLANTAGO LANCEOLATA Lin. Var. CONTORTA, Guss.

In rich grounds on the banks of the Detroit River, No. 3849½ Sept. 2, 1914.

PLANTAGO LANCEOLATA, Lin. Var. IRRIGUA, Dcne.

Banks of Stoney Creek, No. 3924, Oct. 25, 1914.

PLANTAGO LANCEOLATA, Lin. Var. ERIOPHYLLA Webb.

On stony or sandy grounds in the Keweenaw Peninsula, where it is very common, No. 3916, October 17, 1914; No. 2643, June 9, No. 2763, June 30th, and No. 3017, Aug. 4, 1912, Parkedale Farm.

GALIUM APARINE L. Var. VAILLANTII (D. C.) Koch.

Differing from the species only in its smaller size in all its parts. Farwell, No. 3652, May 30, 1914, in rich muck lands on Parkedale Farm.

SOLIDAGO BICOLOR, Lin.

In Michigan this is a very variable species, but several well defined forms can be distinguished. The stems are simple up to the infloresence, single or several from the same crown. The heads are 2 to 3½ lines high, the involucral scales have a green midrib broadened above, are oblong or obovate, rounded at top and often ciliate; mature achenes several nerved, scabrous on the nerves and with or without a few appressed hairs; the lower cauline and root-leaves vary from obovate or oblanceolate to oval, oblong and lanceolate; cauline leaves below the inflorescence from 12 to 30; the ray flowers are white, cream color, yellow or orange-yellow; the upper leaves are oblanceolate, oblong, or lanceolate, passing into the floral bracts, entire or minutely incurved denticulate; the whole plant is more or less hirsute. The inflorescence is a simple or branched thyrse, more or less inter-

rupted. When all features are considered it seems best to maintain the species intact as was done by Torrey and Gray. The Michigan varieties are as follows:

The typical plant is from 1 to 2 feet high, has the lower cauline and root leaves oblanceolate, 1 to 4 inches long by ½ to 1½ wide, and gradually tapering into a short (1½ inches or less) broadly winged petiole, coarsely dentate; upper cauline ¾ to 1½ inches long, by ¼ to ½ inches wide. Involucral scales greenish, head 2½ lines high, rays white. Inflorescence a slender, interrupted thyrse and globular clusters or short racemes in the axils of the upper leaves; cauline leaves 12 to 24. Fields and hillsides at Rochester. Farwell, No. 3857, Sept. 7, 1914; No. 877, Sept. 27, 1895, Detroit; No. 3534, Oct. 5, 1913, Parkedale Farm.

Solidago bicolor L. var. luteola, N. Var.

Similar; lower cauline and root leaves obovate or oblanceolate, the blade shorter and broader, ¾ to 1½ inches wide by 1½ to 2½ inches in length, tapering into a somewhat longer and more narrowly winged petiole, ¾ to 1½ inches long; upper leaves smaller, an inch or less in length and less than a ¼ inch wide; rays of the color of cream or honey. With the species but less common. Farwell, No. 3801, Sept. 7, 1914.

Solidago bicolor Lin. Var. paniculata, N. Var.

Taller, 2½ feet high; root-leaves oblong or lanceolate 2½-3½ inches long by 1-1½ inches wide, coarsely dentate, acute, tapering into slender, narrowly margined petioles, about twice as long as the blades, which are hirsute on each face with scattered, appressed short hairs becoming more thickly placed as they pass on to the petioles; the cauline leaves about 30, the lower similar to the root leaves in shape but on short petioles 1½ inches or less, the more copious pubescence on the under surface spreading, upper cauline similar but smaller, 1½ by ½ inch or less; inflorescence paniculate, the branches 1 to 4 inches long, virgate, ascending, densely flowered; rays white. The lanceolate, acute leaves and paniculate inflorescence give this form a very distinct appearance. In fields at Algonac. Farwell, No. 3903, Sept. 13, 1914.

Solidago bicolor Lin. var. ovalis, N. Var.

About 2 feet high. Leaves very thin, proportionately broader than in any of the other varieties, the lower cauline and root

leaves oval, 2-3 inches long, 1-1¾ wide, crenate-serrate on petioles of their own length, the upper cauline 2 inches or under and ⅔ of an inch wide or less. Thyrse, simple; rays yellow fading to white. With the species but less common. Farwell, No. 3838, August 23, 1914. In the above varieties of this species the heads are 2½ lines high and the bracts of the involucre are greenish with a pale border, giving the whole thyrse a pale or ash-colored appearance. In the next two varieties the whole thyrse has a yellowish appearance as the involucral bracts are greenish with a yellowish border and the heads are 3 to 3½ lines high.

Solidago bicolor Lin. Var. concolor, Torr. & Gray.

Solidago hispida Muhl.

Solidago hirsuta Nutt.

Similar to S. bicolor Lin. but the rays are a deep yellow and the heads larger. In similar situations; Farwell, No. 3859, Sept. 7, 1914; No. 481a, August 29, 1895, Orion; No. 481b August, 1908, Detroit.

Solidago bicolor Lin. Var. spathulata, N. Var.

One or two feet high, 15 to 18 cauline leaves below the inflorescence; the lowest and the root leaves oblong-spatulate, about 4 inches long by 1½ inches broad near the rounded apex, crenate serrate, rather abruptly cuneately tapering into broadly winged petioles an inch or so long; upper cauline elliptical, 1½ inches or less in length by a third as wide, generally obtuse; heads and rays as in the preceding variety. Farwell, No. 481, Sept. 12, 1886. Generally on rocky, sandy or poor soil, Keweenaw Peninsula.

Solidago graminifolia (Lin.) Salisb. Var. Nuttallii (Greene) Fern.

The more hispidulous pubescent variety of the species. Farwell, Nos. 3871, Sept. 17, 1914, Parkedale Farm; 484a, Aug. 31, 1892, Belle Isle. Probably the common form in the southern part of Michigan.

Aster puniceus, Lin. Var. demissus, Lindl.

A form in which the leaves are longer than their axillary inflorescences. Rochester. Farwell, No. 3865, Sept. 7, 1914.

Helianthus giganteus Lin. Var. virgatus (Lam.), N. Comb.

Helianthus virgatus Lam. Encyl. 3, 85, 1789.

Helianthus altissmus Var. *virgatus* Pers. Syn. 2, 476, 1807.

Stem slender, simple, 4 feet or less high, terminated by a single head with one or two additional in the axils of as many of the uppermost leaves; leaves opposite, except the uppermost, very thin and delicate, lanceolate, coarsely serrate, green, nearly of the same hue on both faces; pubescence copious on the upper parts, much as in the species, but *white*, not rusty. Swamps. Farwell, No. 1623c, Aug. 23, 1914, Stoney Creek; No. 1623b, Sept. 2, 1901, Lakeville; No. 1623a, Aug. 20, 1892, Belle Isle.

Helianthus giganteus Lin. Var. oppositifolius, N. Var.

Stems three feet or under; leaves mainly opposite, some conduplicate, firm, narrowly lanceolate, acuminate at both ends, 4 or 5 inches long or less, bluish green, paler below; heads few, sometimes only one, when more, terminating axillary branches, forming a simple 3-6-flowered corymb; pubescence appressed, only on the uppermost parts. Fields at Algonac. Farwell, No. 3884, Sept. 13, 1914; No. 1623, Sept. 27, 1898, Birmingham. Bears about the same relation to H. giganteus as H. Maximiliani Var. Dalyi (Britton), N. comb. (*Helianthus Dalyi* Britt. Journ. N. Y. Bot. Gard. 2, 89, 1901) does to H. Maximiliani. Bears slender fusiform rhizomes or these ending in small tubers.

Helianthus giganteus Lin. Var. altissimus (Lin.), N. Comb.

Helianthus altissimus Lin. Sp. Pl. Ed. 2, II, 1279, 1863.

Like the species but glabrous and glaucous on the stem, the branches and peduncles sparsely appressed hirsute; the tips of the chaff in this variety are not black as in the species; No. 3883, Sept. 13, 1914, Algonac.

Helenium autumnale Lin. Var. grandiflorum (Nutt.) T. & G.

In addition to the typical form with narrow-lanceolate, entire or denticulate leaves there is another with broadly lanceolate leaves that are coarsely serrate or dentate; the achenes are shorter, broader and wider above than in the former and the pappus scales are smaller and longer awned. It seems to be Torrey and Gray's Var. grandiflorum. Farwell, No. 3874, Sept. 7, 1914, Parkedale Farm.

Hieracium florentinum All.

This was found in fields near Calumet and Lake Linden. One

of the plants that is known in the Eastern States as King Devil. Farwell, No. 3915, Oct. 11, 1914.

Corrections in Nomenclature.

In rearranging the plants in my herbarium I found it necessary, in order to give them the correct names that they should have, to make some new combinations which may be recorded here.

Elodea minor (Engelm) N. Comb.

Udora verticillata? Var. *minor* Engelm; Casp. Pahrb. Wiss. Bot. 1, 654, 1858.

Muhlenbergia brevifolia (Nutt.), N. Comb.

Agrostis brevifolia Nutt. Gen. 1, 144, 1818.

Vilfa cuspidata Torr.; Hook, Fl. Bor. Am. 2, 238, 1840.

Muhlenbergia cuspidata Rydberg in Bul. Torr. Bot. Cl. 599, 1905.

Sporobolus brevifolius Scribn. Mem. Torr. Bot. Cl. v. 39, 1895, p. p.

Muhlenbergia Mexicana (Lin.) Trin. Var. commutata (Scribn.), N. Comb.

Muhlenbergia Mexicana (Lin.) Trin. Subsp. *commutata,* Schribn. Rhodora, 9, 18, 1907.

Reboulea, Kunth, Rev. Gram. 1, 341, Pl. 84, 1830.

In Rhodora for August, 1906, Mr. Scribner in a discussion of the grasses then known as Eatonia points out that they are without a generic name and so creates a new genus Sphenopholis to include them and some others from Trisetum. He passes by Reboulea Kunth 1830 on account of Reboulea Raddi 1820, a genus of Liverworts (Hepaticae). But Mr. Scribner is in error as Raddi's genus is *Rebouillia,* a name that cannot conflict with Reboulea Kunth nor cause confusion in any way by maintaining both as valid genera. Nees ab Essenbeck in 1846 changed Raddi's genus to Reboulia, but as this was 16 years after Kunth's genus was established it cannot be accepted as valid; the original spelling of the hepatic genus must therefore be maintained, and Reboulea Kunth is valid for the Eatonias. Michigan forms are:

Reboulea obtusata (Mx.) A. Gr. Var. pubescens (S. & M.), N. Comb.

Eatonia pubescens, Scribn. & Merr. Circ. U. S. Dep. Agr. Agrost. 27, 6, 1900.

REBOULEA PALLENS (Spr.), N. Comb.

Aira pallens Spr. Fl. Hal. Mant. 33, 1807.

REBOULEA NITIDA (Spr.), N. Comb.

Aira nitida Spr. l. c. 32.

REBOULEA NITIDA VAR. GLABRA (Nash.), N. Comb.

Eatonia glabra Nash. In Britt. Man. 1043, 1901.

Other species and varieties not already transferred are as follows:

(*Sphenopholis palustris* Scrib. and allied species will be better taken care of if allowed to remain in Trisetum.)

REBOULEA OBTUSATA var. LOBATA (Trin.), N. Comb.

Trisetum lobatum Trin. in Mem. Acad. Petersb. Ser. 6, 1, 66, 1831.

REBOULEA FILIFORMIS (Chapm.), N. Comb.

Eatonia Pennsylvanica var. *filiformis* Chapm. Fl. S. St. 560, 1860.

REBOULEA PALLENS var. LONGIFLORA (Vasey), N. Comb.

Eatonia longiflora (Vasey) Beal, Grasses N. Am. 2, 494, 1896.

REBOULEA PALLENS var. MAJOR (Torr.), N. Comb.

Koeleria tunicata var. *major* Torr. Fl. N. & M. U. S. 117, 1824.

ERAGROSTIS CAPILLARIS var. FRANKII (Steud.), N. Comb.

Eragrostis Frankii Steud. Syn. Pl. Gram. 273, 1855.

More robust and more freely branched than in the species, with shorter panicle branches.

ERAGROSTIS ERAGROSTIS var. MEGASTACHYA (Koeler), N. Comb.

Eragrostis vulgaris a *megastachya* Coss u Germ. Fl. Paris 2, 641, 1845.

ERAGROSTIS PILOSA var. CAROLINIANA (Spr.), N. Comb.

Poa Caroliniana Spr. Fl. Hal. Mant. 33, 1807.

Eragrostis Purshii Schrad. Linnaea, 12, 451, 1838.

POTAMOGETON GRAMINEUS Lin. Pl. 1, 27, 1753.

Potamogeton gramineus A *graminifolius* Fries. Nov. Fl. Suec. 36, 1828, and a *fluvialis,* l. c. 37.

Floating and submerged leaves alike, sessile or subsessile.

POTAMOGETON GRAMINEUS var. LACUSTRIS (Fries), N. Comb.

Potamogeton gramineus A *graminifolius b lacustris* Fries l. c. 37.

Similar to the next but the floating leaves are membranaceous, not coriaceous.

POTAMOGETON GRAMINEUS var. PARVIFOLIUS (Nolte), N. Comb.

Potamogeton heterophyllus var. *parvifolius* Nolte in Wallr. Shed. 1822, ex Fries, l. c.

Potamogeton gramineus B *heterophyllus* and d *stagnalis* Fries, l. c.

Potamogeton heterophyllus Wallr. Sched. Crit. 1, 64, 1822, and Amer. Authors not Schreb according to Fries.

Floating leaves coriaceous, petioled, elliptic, ovate, rounded at base, shorter and broader than the submersed leaves.

ERIOCAULON AQUATICUM (Hill) G. C. Druce Pharm. Journ. 29, 700, 1909, should be adopted for *E. septangulare,* With.

A FREAK OF NATURE.

By O. A. Farwell.
(Department of Botany, Parke, Davis & Company, Detroit, Mich.)

While walking through Algonac, Michigan, I observed a peculiar freak of nature which may prove of interest to readers of Torreya. Two trees of *Populus alba* were growing side by

Fig. 1. *Populus alba*. "Siamese Twins."

side, the lower parts in close contact, and were located on a residential corner lot. The freak consists of a small branch, eight or nine inches in diameter, of one tree piercing the trunk of the other, completely passing through and showing on the other side; the branch passes through the trunk a little to one side of its center, bridging the space between the two trees. The branch extended several feet beyond the pierced trunk, but ultimately died. Two photographs were taken just after the pruning of the tree had been accomplished, and in the one reproduced on page 115 the ladder used in the operation is seen still resting

against the bole of the tree. The butt is four inches in diameter and appears to be emerging from both the bole and its branch. The probable explanation of this condition is that when both trees were young, a branch of one crossed a branch of the other, resting firmly in the axil or crotch formed by the bole and its branch; the friction caused by the natural growth of the parts involved, assisted by winds and storms, probably wore away the barks until the cambium layers were reached, when a union of these tissues took place, resulting ultimately in completely covering the intruding branch with wood and bark of the other tree, bringing about the present appearance which might be likened unto that of "Siamese Twins." This process resulted in the gradual strangulation, cessation of growth, and death of that part of the branch beyond the tree; the other part or "bridge" continued its growth and its union with the tree is so perfect that its bark is as normally continuous with that of the pierced trunk as with that of the parent tree.

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FERN NOTES.

BY OLIVER ATKINS FARWELL.

(Department of Botany, Parke, Davis & Company, Detroit, Mich.)

During the past few years as a result of researches in field, herbarium, and library, a number of interesting discoveries and novelties have been brought to light and this paper puts on record some of the results and conclusions arrived at during the course of these studies.

POLYPODIALES.

POLYPODIACEAE.

Pteris aquilina, Linne var. Pseudocaudata, Clute.

This is a form of the species in which many of the pinnules are *narrow, entire,* and *elongated,* particularly the terminal ones. It is only rarely met with. I have found it at Detroit, No. 3516½, August 10, 1913, in sterile or sandy situations; also on sandy hills at Rochester, No. 2560½, July 14, 1912.

Asplenium pinnatifidum, Nutt.

I have never seen this species in the field, but in my herbarium I have a sheet showing several plants which were collected at Cobden, Illinois, by Mr. M. B. Waite, June 8, 1885. These, with the exception of one plant, are normal A. pinnatifidum; the one abnormal plant is normal in all respects except segmentation, which is exactly that of A. ebenoides, R. R. Scott, *i.e.*, the lobes are lanceolate and acute instead of round-ovate and obtuse, and of variable lengths, short and long lobes often alternating. If A. ebenoides is a hybrid between Camptosorus rhizophyllus and Asplenium platyneuron with a trend toward the latter parent, why may not A. pinnatifidum be a similar hybrid with a tendency toward the former parent? This peculiar plant would seem to so indicate.

Asplenium platyneuron (Linne) Oakes.

A rare fern in Michigan. Beal, in the Michigan Flora, states that Allegan is the only station in the State. I found it at Williamstown, Ingham Co., May 28, 1905, No. 1903.

Athyrium Felix-femina (Linne) Roth.

There is a wide degree of variation in the pinnation and size of the different forms that have been referred to this species; the extremes have been variously regarded as synonymous with the typical form, as varieties of it, or as distinct species. Since the indusial characters, texture of fronds, and general appearance are much the same in all the forms the happiest medium probably will be best served by considering them all as varieties of one species. In addition to the type the following varieties are found in Michigan.

ATHYRIUM FILIX-FEMINA var. MICHAUXII (Spreng.), N. Comb.

Aspidium angustum Willd., Sp. Pl., 5, 277, 1810.

Asplenium Michauxii Spreng., Syst. 4, 88, 1827.

Asplenium Filix-femina var. Michauxii Mett., Fil. Hort. Lips., 79, 1856.

Athyrium asplenoides var. *angustum* Moore, Index, 179, 1860.

Asplenium Filix-femina var. *angustum* D. C. E. Ferns of the South-west, 330, 1878.

Athyrium Filix-femina var. *angustum* (Willd.) Farwell, Mich. Acad. Sci., 6, 201, 1904.

Keweenaw Co., No. 757, July 18, 1890. Frequent in rocky situations. Parkedale Farm, No. 3039a, August 4, 1912. Frequent in dry thickets.

Athyrium Felix-femina var. multidentatum (Döll) Milde, Fil, Eur., 50, 1867.

Asplenium Felix-femina var. *multidentatum* Döll, Rhein. Fl., 12, 1843.

Athyrium Felix-femina var. *cyclosorum* (Ruprecht) Moore, Index, 183, 1860.

The largest and most divided form. Keweenaw Co., No. 502. July 28, 1887, in moist thickets; common. Detroit, No. 502a, Oct. 16, 1910, in moist thickets; common.

ATHYRIUM FILIX-FEMINA VAR. LATIFOLIUM, Moore, Nat. Pr. Brit. Fer. t. 31B, 1855.

Keweenaw county; No. 590, Sept. 5, 1887, in rocky or sterile situations; frequent.

FILIX (Fuchs) Hill, Family Herbal 171, 1755.

Dryopteris Adanson, Fam. Pl. 2, 20 and 550, 1763.

Aspidium Swartz, Schrad. Journ. Bot. 1800, 2, 29, 1801.

Nephrodium Rich., Cat. Jard. Med. Par. 120, 1801.

Lastrea Bory, Dict. Class. d'Hist. Nat. 6, 588, 1824.

Underwood and others have adopted *Filix,* Adanson (1763), as the oldest post Linnaean name for those ferns that generally have been known under the name of *Cystopteris,* Bernhardi (1806). According to Christensen, Ludwig used the name *Filix* in 1757, perhaps in the same sense. Hill, however, in the Family Herbal used it for the Male Fern and the Female Fern. I will quote a few lines from the preface of this volume in order to show the attitude at that time of Sir John Hill toward botanical science as well as to show that he intended the volume to be of a botanical nature as well as a medical dispensatory.

"It grieves a man of public spirit and humanity, to see those things which are the means alone of the advantages of mankind studied, while in the end that advantage itself is forgotten. And in this view he will regard a Culpepper as a more respectable person than a Linnaeus or a Dillennius." "That Botany is an useful study is plain; because it is in vain that we know botany is good for headaches, or self-heal for wounds, unless we can distinguish betony and self-heal from one another, and so it runs through the whole study."

"We are taught by it to know what plants belong to what names, and to know that very distinctly; and we shall be prevented by that knowledge from giving a purge for an astringent, a poison for a remedy; let us therefore esteem the study of botany, but let us know, that this use of the distinctions it gives is the true end of it; and let us respect those, who employ their lives in establishing those distinctions upon the most certain foundations, upon making them the most accurately, and carrying them the fartherest possible; these are the botanists; but with all the gratitude we owe them for their labours, and all the respect we show them on that consideration, let us understand them as but the seconds in this science. The principals are those who know how to bring their discoveries to use, and can say what are the ends that will be answered by those plants, which they have so accurately distinguished."

"The plants are arranged according to the English alphabet, that the English reader may know where to find them: they are called by one name only in English, and one in Latin; and these

are their most familiar names in those languages; no matter what Casper or John Bauhine, or Linnaeus call them, they are here set down by those names by which every one speaks of them in English; and the Latin name is added, under which they will be found in every dictionary. To this is subjoined a general description of the plant, if it be a common one, in a line of two; that those who already know it, may turn at once to the uses; and for such as do not, a further and more particular account is added."

There is, then, no doubt that he intended the work to be botanical as well as useful from a therapeutic point of view, and it can not, therefore, be ignored any more than other volumes of a botanical nature. The Latin names are either uninomials, binomials, or polynomials. The work contains no generic descriptions as such, but the Latin names are accompanied by descriptions supplemented, in some instances, by illustrations, so that there is no question as to the identity of the plant described, thus making the publication effective according to Article 35 of the Vienna Code. On pages 171 and 172, in the order given herewith, Hill described two species: Male Fern, *Filix mas,* and Female Fern, *Filix Foemina.* The Male Fern is the species known as such at the present time. The Female Fern is the one that was published by Linne as *Pteris aquilina.* The names *Filix mas* and *Filix foemina* as here used by Hill must be considered as true binomials and not in any sense as generic names as employed by him a year later in the British Herbal. Since the binomial has been effectively published it follows that each element of the binomial, that is to say, that the generic name and the specific name each has been effectively published and the proper citation for the genus is Filix (Fuchs) Hill, Family Herbal 171, 1755.

The North America species not already transferred are as follows:

Filix ampla (H. & B.), N. Comb.

Polypodium amplum H. & B. ex Willd., Sp. Pl., 5, 207, 1810.

Filix aquilonaris (Maxon), N. Comb.

Dryopteris aquilonaris Maxon, Bul. Tor. Bot. Cl., 27, 638, 1900.

Filix Bootth (Tuckerm.), N. Comb.

Aspidium Boottii Tuckerm., Hovey's Magazine, 9, 145, 1843.

Filix cristata (Linne), N. Comb.

Polypodium cristatum Linne, Sp. Pl., 1090, 1753.

Filix cristata var. Clintoniana (D. C. E.), N. Comb.

Aspidium cristatum var. *Clintonianum* D. C. E. in Gr. Man., Ed. 5, 665, 1867.

Filix floridana (Hook), N. Comb.

Nephrodium Floridanum Hooker, Fil. Exot., t. 99, 1859.

Filix fragrans (Linne), N. Comb.

Polypodium fragrans Linne, Sp. Pl., 1089, 1753.

Filix goggilodes (Schk.), N. Comb.

Nephrodium unitum R. Br., non Sieb., nor *Polypodium unitum* Lin., Syst. Nat., X., 2, 1326, 1759.

Aspidium goggilodus Schk., Kr. Gew., 1, 193, t. 33c, 1809.

Filix Goldiana (Hooker), N. Comb.

Aspidium Goldianum Hooker, Edinb. Philos. Journ., 6, 333, 1822.

Filix Goldiana var. celsa (Palmer), N. Comb.

Dryopteris Goldiana celsa Palmer, Proc. Biol. Soc. Wash., 13, 65, 1899.

Filix marginalis (Linne), N. Comb.

Polypodium marginale Linne, Sp. Pl., 1091, 1753.

Filix marginalis var. bipinnatifida (Clute), N. Comb.

Nephrodium marginale f. bipinnatifidum Clute, Fern Bul. 19, 50, 1911.

In woods at Detroit No. 1652, August 22, 1899, rare. This fern has the general appearance of F. spinulosa var. Americana, but it is not spinulose and the sori are marginal. It apparently is the same thing described by Clute as Nephrodium marginale forma bipinnatifidum. It may be one of the so-called fern hybrids with Filix marginalis and F. spinulosa var. Americana as the parents.

Filix montana (Vogler), N. Comb.

Polypodium montanum Volger, Dissert, 1781.

Polypodium oreopteris Ehrh. ex. Willd., Prod., 292, 1787.

Filix Noveboracensis (Linne), N. Comb.

Polypodium Noveboracense Linne, Sp. Pl., 1091, 1753.

Filix opposita (Vahl), (*Polypodium oppositum* Vahl, Ecl. Amer., 3, 53, 1807) var. strigosa (Fee), N. Comb.

Aspidium strigosum Fee, 11 Mem., 78, t. 22, f. 2, 1866.

Dryopteris contermina strigosa (Fee) Underwood.

FILIX OREGANA (C. Chr.), N. Comb.

Dryopteris Oregana C. Chr., Ind. Fil., 281, 1905.

FILIX PARASITICA (Linne), N. Comb.

Polypodium parasiticum Linne, Sp. Pl., 1090, 1753.

FILIX PATENS (Swartz), N. Comb.

Polypodium patens Swz., Prod., 133, 1788.

FILIX PATENS var. STIPULARIS (Willd.), N. Comb.

Aspidium stipulare Willd., Sp. Pl., 5, 239, 1810.

FILIX PATULA (Swartz), N. Comb.

Aspidium patulum Swz., Vet. Ak. Hdl. (1817), 64.

FILIX RIGIDA (Hoffm.) (*Polypodium rigidum* Hoffm., Deutsch. Fl., 2, 6, 1795) var. arguta (Klf.), N. Comb.

Aspidium argutum Klf., Enum., 242, 1824.

FILIX SETIGERA (Blume), N. Comb.

Cheilanthes setigera Blume, Enum., 138, 1828.

FILIX SPINULOSA (Muell.) Farwell var. AMERICANA (Fischer), N. Comb.

Aspidium spinulosum Americanum Fischer ex. Kunz, Amer. Jour. Sci., Ser. 2, 6, 84, 1848.

FILIX SPINULOSA var. CONCORDIANA (Davenp.), N. Comb.

Dryopteris spinulosa (Muell.) Swz. var. *Concordiana* (Davenp.) Eastman, New England Ferns, 1904, and in Gray's New Man., 43, 1908.

FILIX SPINULOSA var. DILATATA (Hoff.), N. Comb.

Polypodium dilatatum Hoff., Deutsch. Fl. 2, 7, 1795.

The *F. spinulosa* var. *dilatata* Farwell, Mich. Acad. Sci., 6, 209, 1904, is the var. Americana.

FILIX SPINULOSA var. INTERMEDIA (Muhl.), N. Comb.

Polypodium intermedium Muhl. ex. Willd., Sp. Pl., 5, 262, 1810.

FILIX SPINULOSA var. PITTSFORDENSIS (Slosson), N. Comb.

Dryopteris Pittsfordensis Slosson, Rhodera, 6, 75, 1904.

Cystopteris Filix-fragilis (Lin.) Chiovenda.

A common fern in rocky woods. Besides the typical form three others are frequently met with.

CYSTOPTERIS FILIX-FRAGILIS var. LOBULATO-DENTATA (Koch), N. Comb.

C. fragilis var. *lobulato-dentata* Koch., Syn., Ed. 2, 980, 1845.

C. fragilis var. *dentata* Hooker, Sp. Fil., I, 198, 1846.

C. Filix-fragilis var. *tenuis* (Mx.) Farwell, Mich. Acad. Sci., 6, 200, 1904.

The earliest varietal name is that of Koch.

Keweenaw Co., No. 830, August 30, 1890, in rocky woods. Frequent. Ypsilanti, No. 830a, June 11, 1892, in moist woods.

CYSTOPTERIS FILIX-FRAGILIS var. ANGUSTATA (Hoff.), N. Comb.

Polypodium fragilis var. *angustatum* Hoff., Roem. et Uster. Mag., IX, Pt. 11, t. I, Fig. 14d, 1790.

C. fragilis subvar. angustata Koch. Syn., Ed. 2, 980, 1845.

C. fragilis var. *angustata* Luerssen, Farnpfl, 459, 1889.

Keweenaw Co., No. 405½, July 8, 1886, in rocky woods; frequent.

CYSTOPTERIS FILIX-FRAGILIS var. LACINIATA (Davenp.), N. Comb.

C. fragilis var. *laciniata* Davenp. in D. C. E., Ferns of N. Amer., 2, 52, 1880.

Keweenaw Co., No. 830½, August 30, 1890, in rocky woods; rare.

These forms or varieties are well illustrated on Plate 53 of Eaton's Ferns of N. America.

OPHIOGLOSSACEAE.

OPHIOGLOSSUM VHLGATUM, Lin.

A variable species which, taken as a whole, has an equally variable habitat. I have found it in Keweenaw Co., but it is not frequent even when met with. The typical species has a sessile sterile frond near the middle of the stem, about equalling the fertile segment, or sometimes a little longer or a little shorter. No. 584½, Sept. 5, 1887, in moist, sandy places along the borders of shallow streams.

OPHIOGLOSSUM VULGATUM var. PSEUDOPODUM (Blake), N. Comb.

Ophioglossum vulgatum forma *Pseudopodum* Blake, Rhodora, 15, 87, 1913.

A larger plant than the species, the sterile frond more ovate, ½ to 1¼ inches wide by 3 to 5 inches long, and tapering into a

petiole like base. No. 584, Sept. 5, 1887, in wet meadow lands with more or less sphagnum and other mosses.

Ophioglossum vulgatum var. minus, Moore.

This is the slenderest form of the species as found in Keweenaw Co. The sterile blade is small (¼ to ⅝ inch wide by ¾ to 1⅜ inches long) ovate or elliptic, sessile near the base of the stalk and far overtopped by the fertile segment, the whole plant about 5 inches in height. No. 585, Sept. 5, 1887, on sterile hillsides covered with a sparse growth of grasses and sedges. The whole plant is yellowish, while that of the other two varieties is green. Undoubtedly this plant belongs here, but it is the one that has been reported in Beal's Flora of Michigan as O. Engelmanni.

Botrychium lunaria var. onondagense (Underwood), N. Comb.

Botrychium Onondagense Underwood, Bul. Torr. Bot. Cl., 30, 47, 1903.

No. 1787, August, 1902, at Copper Harbor in oak and maple woods. Rare. Forms are found which are intermediate between B. Lunaria and B. Onondagense, indicating that the latter is only an extreme form and therefore is better considered as a variety of the former.

Botrychium lanceolatum var. angustisegmentum, Pease and Moore.

The plant listed in Beal's Flora of Michigan as Botrychium lanceolatum is the one recently described as the variety angustisegmentum by Pease and Moore. It grows with B. Matricariaefolium and other forms appear to be intermediate and to intergrade into either; further study may show that it is not specifically distinct from B. Matricariaefolium. No. 588, Sept. 5, 1887; usually in mould under hazel bushes, etc. ,but sometimes in grassy places in the open.

Botrychium Matricariaefolium, A. Br.

It has been very conclusively shown that the Osmunda ramosa, Roth, is not this species and that when Ascherson transferred Roth's specific name to it, it was through a misidentification and resulted in a misapplication of the name. The American plant can not be considered as specifically different from that of Europe. The sterile frond is extremely variable as to the degree of dissection and this fact has led to the description and naming

of several varieties or species based on the degree of division of the sterile lamina. It is a very common fern in Keweenaw Co., delighting mostly in a rich humus, consisting of moulding and decaying leaves, underneath deciduous shrubs and trees but not disdaining to come out into the open, where it may be found in grassy patches, when it is almost completely hidden from view. I have seen large colonies of it, and almost every form imaginable is to be found in such a colony; this fact alone proves that the various forms are of one and the same species. The typical form has the sterile blade oblong or ovate, simply pinnate with the more or less distant pinnae lobed or pinnatifid, the lowest pair somewhat longer than the others. No. 1612a, August 25, 1898, Keweenaw Co.

Botrychium matricariaefolium A. Br. var. rhombeum (Angstrom), N. Comb.

Botrychium Lunaria var. *rhombeum* Angstrom, Bot. Not., 70, 1854.

Botrychium Matricariaefolium var. *subintegrum* Milde, Mon. der deutchs. Ophioglos. 14, 1856.

Botrychium ramosum var. *neglectum* (Wood) Farwell, Mich. Acad. Sci., 6, 200, 1904.

This is a simple form of the sterile frond which is 1 or 2 inches long, simply pinnate with 3-9 nearly equal, rounded, oval, or oblong, obtuse, pinnae, more or less toothed or incised. No. 618, July 26, 1888, in moist shady woods in Keweenaw Co. No. 2714, June 16, 1912, in open, moist, sandy fields, near Algonac.

Botrychium Matricariaefolium var. compositum Milde.

This variety has the lowest pair of pinnae much elongated and pinnate so that the whole frond appears to consist of three subequal and similar divisions. No. 1612, August 22, 1898, in maple and oak woods in Keweenaw Co.

Botrychium dissectum Spreng., Anleit., 3, 172, 1804.

Botrychium lunarioides var. *dissectum.* A. Gr., Man. Bot., 635, 1848.

Dissectum is the earliest specific name for that group of forms that has been passing as Botrychium obliquum and hence Sprengel's name should be restored. The ultimate divisions are ovate or oblonglanceolate, incisely toothed. In moist thickets and fields, Detroit, rare. No. 1975, June 18, 1906.

Botrychium dissectum var. obliquum (Muhl.), N. Comb.

Botrychium obliquum Muhl. ex Willd., Sp. Pl., 5, 63, 1810.

Botrychium lunarioides var. *obliquum* A. Gr., Man. Bot., 635, 1848.

The ultimate divisions are crenulate-serrulate. In fields and more frequent than the type. No. 872, October 15, 1895, at Detroit.

Botrychium dissectum var. elongatum (Gilbert & Harberer), N. Comb.

Botrychium obliquum var. *elongatum* Gilbert & Harberer, Fern Bul., 11, 89, July, 1903.

Ultimate segments lanceolate, elongated, crenulate-serrulate. Occasional, No. 3552½, October 12, 1913, in sandy fields at Algonac.

Botrychium multifidum (Gmel.) Rupr.

Osmunda multifida Gmel., Nov. Comm. Ac. Petr., 12, 517, t. 11, f.1, 1768.

Osmunda Matricariae Schrank, Bair. Flora, 2, 419, 1789.

Botrychium Rutaefolium A. Br. ex. Döll, Rhein. Flora, 24, 1843.

Botrychium ternatum \ *Europaeum* Milde, Fil. Europ., 199, 1867.

Botrychium ternatum var. *Rutaefolium* D. C. E., Fer. N. Amer., 1, 149, 1879.

This species is similar to the last preceding, but it is usually larger, more compound in most of its forms, with the ultimate segments ovate or obovate and obtuse. The type is rather a small plant with few, broad ovate, obtuse segments, the lowest sublunate. No. 627, July 31, 1888, Keweenaw Co., in moist, sandy places; No. 2715, June 16, 1912, near Algonac.

Botrychium ternatum var. Oneidense (Gilbert), N. Comb.

Botrychium obliquum var. *Oneidense* (Gilbert) Waters in Gray's New Manual, 49, 1908.

The broadly oblong, obtuse, sub-cordate segments of this variety seem to place it with this species rather than with the preceding. Keweenaw Co., No. 854, July 5, 1895, in moist meadows.

Botrychium multifidum var. australe (D. C. E.), N. Comb.

Botrychium ternatum var. *australe* D. C. E., Ferns N. Amer., 1, 149, Plate XX a (largest plant), 1879.

Botrychium silaifolium Pr., Rel. Haenk, 1, 76, 1825.

Botrychium occidentale Und., Bul. Torr. Bot. Cl., 25, 538, 1898.

Botrychium obliquum var. *Harbereri* Gilbert.

This is the largest form of the species and many individuals carry the sterile lamina of the preceding year well along into the summer so that it may be gathered in good condition with two sterile fronds on the same plant. Keweenaw Co., No. 708, Sept. 20, 1888, common in grassy fields and meadows; Rochester, No. 628 a, Aug. 15, 1909.

Botrychium multifidum var. intermedium (D. C. E.), N. Comb.

Botrychium ternatum subvar. *intermedium* D. C. E., Ferns, N. Amer. 1, 149, Plate XX a (Plant in front), 1879.

Intermediate between the species and the variety australe. Fields and meadows, common, Keweenaw Co., No. 628, July 31, 1888.

Botrychium multifidum var. dichotomum, N. Var.

Twice dichotomously branched, showing two long, and one short-stalked, fertile segments and one short-stalked sterile lamina. The primary and secondary divisions of the stem are about 1 cm. in length, while the tertiary divisions are of variable lengths. The sterile lamina is small (15 mm. long by 10 mm. wide at the base), ovate, pinnatified with 5-7 small, closely placed, semilunate to obovate, somewhat cuneate, obtuse lobes, entire or denticulate, on a stalk 1 cm. long; the fertile segment is bipinnate on a stalk 25 mm. long; the other two fertile segments are tripinnate on stalks about 10 cm. in length. This curious plant (Fig. 13) was collected in sphagnum moss and may be a monstrosity, but seems to answer to the state found by C. J. Sprague, at Hingham, Mass., as mentioned in Gray's Manual, 5th Ed. p. 672. Apparently this differs from the Sprague plant in having the long-stalked fertile segments, which represent the lateral divisions of the sterile lamina, arising from low down on the common stalk instead of at the normal positions for those divisions. Keweenaw Co., No. 627a, July 31, 1888.

Botrychium simplex, E. Hitch.

This is a very small plant, and in the field easily overlooked. The typical form has a small, sterile frond, simple, or three-lobed,

Figure 13. Botrychium multifidum var. dichotomum

roundish, or obovate. It is usually found in low wet grounds with, or in the vicinity of, moss. Keweenaw Co., No. 3997½, July 3, 1915.

Botrychium simplex var. angustum, Milde.

Botrychium tenebrosum A. A. Eaton, Fern Bul., 7, 8, 1899.

This variety has a narrow, pinnate, sterile frond with 2 or 3 pairs of distant lobes. It is more frequently found in rich, moist thickets and is liable to be confused with slender and delicate plants of B. Matricariaefolium, with which it is sometimes found in company. Keweenaw Co., No. 644 a, August 8, 1888.

Botrychium simplex var. subcompositum, Lasch.

The sterile lamina is pinnate with 3-5 pairs of contiguous lobes, or with the lower pair remote and narrowed to petiole-like bases. In wet, mossy fields or meadows. Keweenaw Co., No. 644, August 8, 1888.

LYCOPODIALES.

LYCOPODIACEAE.

When on the Keweenaw Peninsula in October, 1914, the season being most propitious for the work, I made a thorough study of the Club Mosses of the region. Among other things observed was the propensity of species of the section Lepidotis to produce proliferous spikes, *i.e.*, spikes with the axes prolonged as leafy shoots; the length of the peduncles is very variable even on the same plant; sometimes the peduncle is obsolete so that the pedicles of the spikes spring from the apex of the branchlet, thus appearing as peduncles. It is customary to consider L. dendroideum, Michaux, as synonymous with L. obscurum, Linne, even though the former has terete branchlets with equal, 8 ranked leaves, while the latter has dorsiventral branchlets and unequal, 4 ranked leaves. So long as this attitude is maintained there is no excuse for keeping L. alpinum separate from L. complanatum as exactly the same conditions prevail. In the living plants of these species the tips of the leaves of the upper and lower rows of the dorsiventral branchlets are never appressed, as is usually stated in our manuals to be the case. The stems creep along the surface or at various depths down to six inches; these with the branches are always terete and bear equal 8-ranked leaves, the free portions of which are never appressed.

Lycopodium Selago, Lin var. patens (Beauv.) Desv.

This variety, as well as the typical species, is rather scarce on the Keweenaw Peninsula; the plant is greener than the species, which is yellowish, and coarser; the leaves are narrow, more sharply pointed, and horizontal or nearly so. In wet, mossy grounds, No. 3910½. October 1, 1914.

Lycopodium clavatum, Lin. var. megastachyon, Fern. and Biss.

The form listed in Beal's Michigan Flora as the var. monostachyon, Hooker, is that plant which has more recently been described by Fernald and Bissel as L. complanatum var. megastachyon. This name should therefore be adopted for the plant found in northern Michigan, as it is very distinct from Hooker's variety.

Lycopodium obscurum, Linne.

Our local manuals describe Lycopodium obscurum Linne as with 6- or 8-ranked leaves with the 2 upper and 2 lower rows appressed. No plant answering to such a description could be found, and it is very doubtful if such a plant can be found anywhere. Linne does not give the number of ranks in which the leaves are arranged but does say that the leaves are spreading (Folia sparsa attamen variora * * * * * * basi decurrentia s. adnata cauli; deni patula). The only reference given by Linnaeus is "Lycopodioides radiatum dichotomum. Dill. musc. 274, t. 67." Dillenius' plate shows a plant that has the leaves in four ranks, the upper row being represented as now appressed and now spreading. Evidently the drawing was made from a dried plant in which naturally enough the upper and lower leaves will most generally appear as appressed. In the living plant the leaves are four ranked on a dorsiventral axis, and ascending with incurved tips, none appressed; the free portion of the lateral leaves is about 4½ mm. in length; of the upper, about 3½; and of the lower, 2. The branches are dichotomously branched, the branchlets ascending with gracefully spreading, recurved tips. Foliage dark green and glossy; perhaps the most graceful and handsome of our Lycopodiums. Fairly well represented by the plate of Dillenius mentioned above. Stems 1 or 2 inches below the surface. Spikes 2-3 cm. Although Linnaeus said he had not seen the fructification of this species, yet, on the other hand, the Dil-

lenian plate referred to by him shows several spikes, most of which are represented with a proliferous tip, a condition very frequently seen in this species.

Another form or variety of this species is the plant known as Lycopodium dendroideum, Mx. It differs much in habit; it is dichotomously branched, as in the specific type, but the branchlets are neither dorsiventral nor drooping, but terete and erect, the upper being shorter, so that the plant has the exact appearance of a miniature spruce tree. The foliage is less glossy and more of a yellowish green in color, the leaves being of equal length, about 3½ mm., and disposed in 8 ranks; the stems are 2 or 3 inches below the surface; the spikes are numerous, sessile, and from 2-5 cm. in length. This will answer very well to Michaux's description. The only reference Michaux gives is Dill. t. 64. The only American species represented on this plate is the Selaginella apoda. Evidently Michaux made a very poor interpretation of the Dillenian plate, if he refers to Dill. Mus. t. 64, or else the reference to it is a typographical error. I have no doubt that this form with 8-ranked, equal leaves, from its remarkable tree-like appearance which is not evident in the other forms of the species, is the plant that Michaux had in view for his L. dendroideum even though that author did not mention the number of ranks in which the leaves are grouped. Most authors attribute six-ranked leaves to Michaux's species, but they evidently have had another variety in hand, one that is exactly intermediate between this plant and L. obscurum, Lin. The branchlets are erect with only the tips slightly curving outward, and are semi-dorsiventral; the leaves are unequal in six ranks, corresponding to three upper and three lower, the latteral row on each side being obsolete; the lower leaves are from 2 to 3½ mm. in length and the upper from 3½ to 4 mm.; the middle upper row bearing the longest leaves, the middle lower row the shortest, while the others are successively intermediate. The stems are from 4-6 inches below the surface. Spikes 2-6 cm. It may be a cross between the other two forms, but it has longer spikes and the stems are deeper in the ground than in either. It may be known as LYCOPODIUM OBSCURUM, Lin. variety HYBRIDUM, N. Var. The species and its synonymy is as follows:

Lycopodium obscurum Lin., Sp. Pl., 1002, 1753.

Lycopodioides radiatum dichotomum. Dill., Musc., 274, t. 67, 1741.

Lycopodium dendroideum var. *obscurum* (Lin.) Torr. ex. Beck., Botany, 460, 1833.

Keweenaw Peninsula, No. 682, September 6, 1888. In rich woods under evergreens. Frequent.

Lycopodium obscurum var. hybridum, Farwell.

Lycopodium Dendroideum Willd., Sp. Pl., 5, 21, 1910, and many American authors, not of Michaux.

Lycopodium obscurum Eaton & Wright, N. Amer. Bot., 309, 1840, and many American authors not of Linnaeus.

Keweenaw Peninsula, No. 3908, September, 1914. Along the edge of woods and thickets. The common form.

Lycopodium obscurum var. dendroideum (Mx.) D. C. Eaton in Gray's Manual, 696, 1890.

Lycopodium dendroideum Mx., Fl. Bor. Amer., 2, 282, 1803.

Keweenaw Peninsula, No. 681, September 6, 1881. On knolls in the open. The rarest form.

Lycopodium complanatum, Linne.

This is a very variable species and its forms have been considered as species by those botanists who think that all variations of plants should be considered as distinct species, discarding all minor categories. This species, like L. obscurum, Linne, shows two well marked series; one with the leaves of equal length and in 6-8 ranks (stems not dorsiventral) and one with the leaves of unequal length and in 4 ranks (stems dorsiventral). The distinctions between L. alpinum asd L. complanatum are not more pronounced than those between L. obscurum and L. dendroideum, yet the former are generally considered as distinct species and the two latter as one and the same thing. As a matter of fact the distinctions are even less pronounced, for L. alpinum shows both kinds of leaves on the same plant while the spikes of L. complanatum may be sessile as in L. alpinum. The extremes appear to be distinct enough, but a complete series of intermediates connect one with the other. L. alpinum has priority of place in the Species Plantarum, but since this species has been reduced to a variety of L. complanatum, the latter, according to Article 46 of the Vienna Rules, must be considered as the type.

Key to the varieties of L. complanatum.

Plants with dorsiventral branchlets, leaves 4 ranked, appressed—in the dried plant.

Branchlets 2-4 mm. wide, very flat, leaves unequal.

Branchlets elongated, loosely ascending.

Peduncles single, 3-12 cm., spikes 2-6—Lycopodium complanatum.

Peduncles, 1 or 2, 1-5 cm., spike solitary—Lycopodium complatum var. Sabinaefolium.

Peduncles obsolete, spike solitary and sessile—Lycopodium complanatum var. Pseudoalpinum.

Branchlets short, crowded, forming funnels (fan-shaped when dried).

Peduncles single, 3-6 cm., spikes 2-6—Lycopodium complanatum var. flabellatum.

Peduncles similar, spike solitary—Lycopodium complanatum var. Wibbei.

Branchlets ½ mm. wide, biconvex, leaves nearly equal.

Leaf tips of lateral rows erect—Lycopodium complanatum var. Chamaecyparissus.

Leaf tips of lateral rows widely spreading—Lycopodium complanatum var. Sharonense.

Plants with both dorsiventral and terete branchlets, leaves 4-ranked, not appressed, spike sessile—Lycopodium complanatum var. alpinum.

Plants with terete branchlets, leaves in 5 ranks, equal, ascending, spikes solitary on peduncles less than 1 cm.—Lycopodium complanatum var. Sitchense.

The species and its more important synonyms are given below.

Lycopodium complanatum, Lin., Sp. Pl., 1104, 1753.

Stems 1-3 inches below the surface. Branchlets elongated, broad and flat, loosely ascending; peduncles 3-12 cm., carrying 2-4, occasionally more, spikes. Keweenaw Co., No. 746, July 12, 1890. Occasional.

LYCOPODIUM COMPLANATUM var. SABINAEFOLIUM (Willd.), N. Comb.

Lycopodium Sabinaefolium Willd., Sp. Pl., 5, 20, 1910.

Lycopodium alpinum var. *Sabinaefolium* (Willd.) D. C. E. in Gray's Man., 696, 1890.

Free portion of leaves longer and narrower, peduncles shorter, solitary, or in twos, spikes solitary, upper leaves often in two rows, the leaves then being 5-ranked, a transition toward var. Sitchense. Stems an inch or so below the surface. Keweenaw Co., No. 746½, July 12, 1890. Rare.

Lycopodium complanatum var. flabellatum, Döll, Fl. Bad., 1, 79, 1855.

Lycopodium anceps Wallr. Linnea, 12, 676, 1840.

Lycopodium complanatum var. *anceps* Aschers., Fl. v. Brand, 1, 894, 1864.

Lycopodium complanatum var. *flabelliforme* Fernald, Rhodora, 3, 280, 1901.

Lycopodium flabelliforme (Fernald) Blanchard, Rhodora, 13, 168, 1911.

This variety is very readily detected in the field by its foliage being arranged in the form of funnels and in herbarium materials by its short, fan-shaped clusters of branches arranged in distinct series one above another. Its stems are above ground. Keweenaw Co., No. 1785 and 1785½, August, 1902; No. 3911 and 3912 (proliferous form), October, 1914.

Lycopodium complanatum var. Chamaecyparissus (A. Br.) Döll, Fl. Bad., 1, 80, 1855.

Lycopodium tristachyum Pursh, Fl. Am. Sept., 653, 1814.

Lycopodium Chamaecyparissus A. Br. ex. Mutel, Fl. Fran., 4, 192, 1837.

Lycopodium complanatum var. *Sabinaefolium* (Willd.), A. Gr., Man., 674, 1867.

The most glaucous form, with the narrowest branchlets, longest peduncles, and most numerous spikes. The commonest form on the Keweenaw Peninsula. Stems 5-6 inches under the surface. No. 686. Sept. 10, 1888.

Lycopodium complanatum var. sharonense (S. F. Blake), N. Comb.

Lycopodium tristachyum var. *Sharonense* S. F. Blake, Fern Bull., 18, 9-10, 1910.

Similar to the preceding but the free portion of the leaves are spreading or recurved. Keweenaw Peninsula, No. 746 a, July 12, 1890. Rare.

Lycopodium complanatum var. alpinum (Lin.), Spring, Flora, 1, 180, 1838.

Lycopodium alpinum Lin., Sp. Pl., 1104, 1753.

The stems are close to the surface; leaves unequal, ascending, in 4 ranks; spikes sessile. Keweenaw Peninula, No. 849, June 30, 1895. Rare.

Three other varieties may be confidentially looked for. These are: var. Wibbei, Harberer, which is similar to the var. flabellatum but with the spike solitary; LYCOPODIUM COMPLANATUM var. PSEUDOALPINUM, N. var., briefly described as like the specific type but with sessile spikes, a transition toward the var. alpinum; and LYCOPODIUM COMPLANATUM var. SITCHENSE (Rupr.), N. Comb. (*Lycopodium Sitchense* Rupr., Beitr. Pfl. Russ., Reich., 3, 30, 1845). Variety Pseudoalpinum is well represented by plate 233, Journal of Botany, Vol. 20, 1882.

NEW RANGES FOR OLD PLANTS.

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In the early autumn of 1915 I was invited by Mr. Gladewitz and Mr. Chandler, both of Detroit, to accompany them on a botanical excursion to Oakwood, a suburban village to the south of Detroit. This is the site of one of the many salt works in this locality. Here is located the Detroit Rock Salt Co., which has, in spite of many difficulties, sunk the only salt "shaft" in Michigan if not in the entire country. The water and fine crystals from the salt works have converted a large tract of land from that of a fresh soil to one of a saline character, which has to a certain extent changed the character of the flora. Many species have disappeared that once were common. Some new ones have crept in that have a distinct preference for saline situations. These are *Salicornia Europaea* L. and its varieties *pachystachya* (Koch) Fernald and *prostrata* (Pall.) Fernald, *Aster subulatus* Mx., and *Pluchea camphorata* (L.) DC. Just how these seaboard plants found their way into Michigan is problematical. Scarcely by means of birds, as the feathered tribes do not travel east and west but rather on a north and south line. We have no substantiated records showing that Atlantic or Pacific birds have migrated across country and into the Great Lake regions. We can only surmise that they may have been brought west by means of railway freight traffic, and when lodgement was made in this section, which provided the proper saline conditions suitable for their development, they persisted and have made flourishing colonies that are rapidly extending over the entire section which has been made saline by means of the escaping water and salt crystals from the mine and the salt crushers. The Rayless Aster and the Salt Marsh Fleabane spread rather slowly but apparently have become firmly established.

The Glasswort has spread very rapidly and now covers acres of ground. The variety *prostrata* with its long, widely spreading, and decumbent lower branches seems very distinct from the normal form of the species with ascending, more uniform (as to

length) branches. The variety *pachystachya* is frequent; but as found here it seems scarcely worthy of recognition. The only distinction is one of measurements, and in the dried specimens even this difference vanishes. There are intermediate individuals also, bearing both thick and slender spikes. Mr. Gladewitz, Secretary and Treasurer of the Detroit Institute of Science, was the first to discover and report these squatters that have pre-empted that section of Michigan territory which seems to provide those cool and saline conditions to which they are accustomed in their native haunts.

Another traveler that recently has been reported from the eastern shores of Michigan between Detroit and Port Huron is *Aster angustus* (Lindl.) T. & G. This is quite plentiful on a common in the village of River Rouge, on the banks of the Detroit and Rouge Rivers. This village is not far removed from Oakwood and the salt fields probably underlie it, though the surface soil, at least to a tyro, gives no indication of a saline character.

Mr. Chandler was the original discoverer of the Rayless Aster at this locality and probably the first to record it from Michigan.

Mr. Billington, of Detroit, has discovered near Palmer Park, Detroit, Mich., a plant that proves to be *Pentstemon gracilis* Nutt. This is far east of its recorded range of from Minnesota to Missouri for its eastern limits. He has also found near Cass Lake *Galium erectum* Huds. This has not been recorded heretofore for localities west of the New England States. It seems to be well established at the locality mentioned. Specimens of the above have been preserved for their private herbaria by the original discoverers. Such as I have collected are as given below.

Salicornia Europaea L.: Oakwood, Mich.; *Farwell, Gladewitz & Chandler,* no. 4105, Sept. 23, 1915. *Salicornia Europaea* Linn. var. *pachystachya* (Koch) Fernald: Oakwood, Mich.; *Farwell, Gladewitz & Chandler,* no. 4107, Sept. 23, 1907. *Salicornia Europaea* Linn. var. *prostrata* (Pall.) Fernald: Oakwood, Mich.; *Farwell, Gladewitz & Chandler,* no. 4103. *Aster subulatus* Mx.: Oakwood, Mich.; *Farwell, Gladewitz & Chandler,* no. 4102, Sept. 23, 1915. *Aster angustus* (Lindl.) T. & G.: River Rouge, Mich.; *Farwell,* no. 4122, Sept. 30, 1915. *Pluchea camphorata* (Linn.) DC.: Oakwood, Mich.; *Farwell, Gladewitz & Chandler,* no. 4104, Sept. 23, 1915.